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Relationships between neuro-endocrine, electrocardiogram, and gastric mucosal damage parameters in naïve and stressed rats

Oksana A. Fihura^{1,2}, Oksana I. Melnyk³, Sofiya V. Ruzhylo¹, Nataliya R. Zakalyak¹, Halyna D. Kondratska¹, Walery Zukow⁴, Igor L. Popovych²

¹Ivan Franko State Pedagogical University, Drohobych, Ukraine oksanafigura08@gmail.com

²Ukrainian Scientific Research Institute of Medicine of Transport, Odesa, Ukraine i.popovych@ukr.net

³Danylo Halytskyi National Medical University, Lviv, Ukraine omelnyk7@gmail.com

⁴Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl

ORCID

OF: <https://orcid.org/0000-0002-5711-0484>

OM: <https://orcid.org/0000-0001-7928-4760>

SR: <https://orcid.org/0000-0003-2944-8821>

NZ: <https://orcid.org/0000-0002-9550-1961>

HK: <https://orcid.org/0000-0001-8856-1125>

WZ: <https://orcid.org/0000-0002-7675-6117>

IP: <https://orcid.org/0000-0002-5664-5591>

Abstract

Introduction

The first scientific publication on general adaptation syndrome, or as we know today biologic stress has been published in Nature in 1936 by the 29-year old Hans Selye.¹ Szabo et al² in the anniversary review "Stress" is 80 Years Old" conclude that despite the extensive and multidisciplinary research on stress during the last 80 years, a lot of basic and clinical research is needed to better understand the

manifestations, central and peripheral molecular regulators of stress response, especially the modes of prevention/management of distress or its transformation into eustress and the treatment of stress-related diseases.

In the vast majority of publications on stress, the HPA-, HPG- and autonomous systems are the objects of research, while the place in the general adaptation syndrome of such important hormones as calcitonin and PTH has been studied only in a few publications. Another methodological shortcoming of most studies is that the subjects of analysis are limited to a single neuro-endocrine system.

Therefore, we set ourselves the goal: to analyze relationships between some adaptation hormones, HRV, calcitonin, and PTH as well as electrocardiogram and gastric mucosal damage in naïve and post stressed rats.

Material and methods

Ethics approval

All animals were kept in room having temperature $22\pm 2^{\circ}\text{C}$, and relative humidity of 44-55% under 12/12 hours light and dark cycle with standard laboratory diet and water given ad libitum. Studies have been conducted in accordance with the rules and requirements of the “General Principles for the Work on Animals” approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001) and agreed with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine “On the Protection of Animals from Cruelty” of 26.02.2006. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Participants

The experiment is at 38 rats Wistar line: 18 males (Weight Mean=227 g; SD=25 g) and 20 females (Mean=214 g; SD=27 g).

Study design and procedure

Due to the purposeful formation of groups, the potential predictors of post-stress reactions of the neuro-endocrine-immune complex and the metabolome^{3,4,5} were almost identical both in mean values and, to a lesser extent, in variance (SD). In particular, the hypoxic test (sec) was: 136 ± 59 and 133 ± 81 ; swimming test (min): 19 ± 11 and 19 ± 17 ; HRV Stress index⁴ (units) as $(AMo/2 * Mo * MxDMn)^{1/3}$: $0,14\pm 0,08$ and $0,14\pm 0,05$ in intact animals and those exposed to acute stress.

Results and discussion

Adhering to the algorithm of the Truskavetsian Scientific School of Balneology, we recalculated the current values of the parameters in Z-scores. This approach makes it possible to adequately compare deviations from the norm of parameters expressed in different units and with different variability.^{10,11,12} This is all the more important in view of sexual dimorphism in endocrine parameters. In addition to drastically higher levels of testosterone (41.8 ± 1.7 vs 3.53 ± 0.24 nM/L), males have higher levels of calcitonin (36 ± 6 vs 21 ± 4 ng/L), but lower levels of parathyroid hormone

(154±12 vs 185±3 ng/L), adrenals mass (44±5 vs 65±5 mg), corticosterone (340±45 vs 466±57 nM/L), and aldosterone (587±8 vs 639±24 pM/L).

Conclusion

The condition of the gastric mucosa and myocardium as essential targets of stressors is determined by the damaging and protective effects of adaptive hormones and the autonomic nervous system.

Keywords. Adaptation hormones, HRV, water-immersion and restraint stress, damage to the gastric mucosa and myocardium, relationships, rats.

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Over the 10 days, one animal remained intact and 3 other rats were exposed to water-immersion and restraint stress (WIRS) according to the method of Nakamura et al.⁶ in the modification of Popovych⁷, which is to reduce the duration of stay of the rat in a fixed standing position in cold water (t⁰ 20-21⁰ C) to the level of the xiphoid process from 8 to 4 hours. Prior to the experiments, rats were fasted for 24 h, but allowed access to tap water *ad libitum*.

The next day after stressing, the ECG under light ether anesthesia was re-recorded in order to assess the state of the myocardium and HRV⁸, and right away the animals removed from the experiment by decapitation in order to remove the stomach, adrenal glands, thymus, spleen, and collect the maximum possible amount of blood in which was determined some endocrine, metabolic, and immune parameters. The last two sets will be the subject of the next article.

Among endocrine parameters determined serum levels of main adaptation hormones such as corticosterone, aldosterone, testosterone, triiodothyronine, as well as parathyroid hormone and calcitonin (by ELISA, with the use of analyzer “RT-2100C” and corresponding sets of reagents from “Alkor Bio”, XEMA Co, Ltd and DRG International Inc).

The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the amount of ulcers and their length was measured, evaluated damage on scale by Popovych (0÷1 points).⁷ This scale is based on the qualitative-quantitative Harrington⁹ scale.

Statistical analysis

Statistical processing was performed using a software package “Microsoft Excell” and “Statistica 6.4 StatSoft Inc” (Tulsa, OK, USA).

First of all, by applying the WIRS model, we reproduced Selye's primary attributes of stress: an increase in adrenal mass, an increase in corticosterone levels, damage to the gastric mucosa and myocardium, on the one hand, and Cannon's attributes: an increase in the level of circulating

catecholamines and sympathetic tone and a reciprocal decrease in vagal tone - on the other hand side (Table 1).

Table 1. Ranking of post-stress changes

| <i>Variables</i> | Intact Rats (10) | Post Stress (28) |
|---|-------------------------|-------------------------|
| AMo HRV as Sympathetic tone, % | 48 6 | +0.88 0.19* |
| Adrenals mass normalized by sex, Z | 0 | +0.51 0.12* |
| 1/Mode HRV as Catecholamines, 1/msec | 1/175 1/10 | +0.47 0.16* |
| Parathyroid hormone normalized by sex, Z | 0 | +0.46 0.22* |
| Aldosterone normalized by sex, Z | 0 | +0.40 0.19* |
| Corticosterone normalized by sex, Z | 0 | +0.28 0.22 |
| Calcitonin normalized by sex, Z | 0 | -0.09 0.16 |
| MxDMn HRV as Vagal tone, msec | 51 14 | -0.59 0.07* |
| Testosterone normalized by sex, Z | 0 | -0.90 0.29* |
| Gastric Mucosal Damage, points | 0 | +1.19 0.20* |
| Gastric Ulcers Amount | 0 | +0.94 0.22* |
| Gastric Ulcers Length, mm | 0 | +0.93 0.19* |
| R wave ECG, μV | 330 18 | +0.90 0.33* |
| P wave ECG, μV | 25 3 | +0.80 0.25* |
| P-Q interval ECG, msec | 55.6 0.8 | -1.88 0.27* |
| T wave ECG, μV | 131 3 | -1.76 0.36* |
| Q-T interval ECG, msec | 104.9 1.2 | -1.07 0.21* |
| S-T joint ECG, μV | 54 5 | -0.79 0.30* |

The moderate expression of the essential signs of stress is explained, firstly, by our deliberate use of mild stressor parameters (water temperature and duration of water immersion), and secondly, by pretreatment of 18 animals with a phytoadaptogen, which, in particular, prevented the post-stress increase in the level of corticosterone. The effects of the adaptogen are not the subject of this study and will be analyzed in the next article, already ready for printing.

We found (Tables 2-3 and Fig. 1) that the number and length of ulcers, as well as the severity of damage (taking into account also the appearance of only petechiae without ulcers) to the gastric mucosa significantly correlate with changes in ECG parameters, in particular, depression of the T

wave and connection S-T, which indicate myocardial dystrophy^{13,14,15,16}, and only weakly with the amplitude of the R wave.

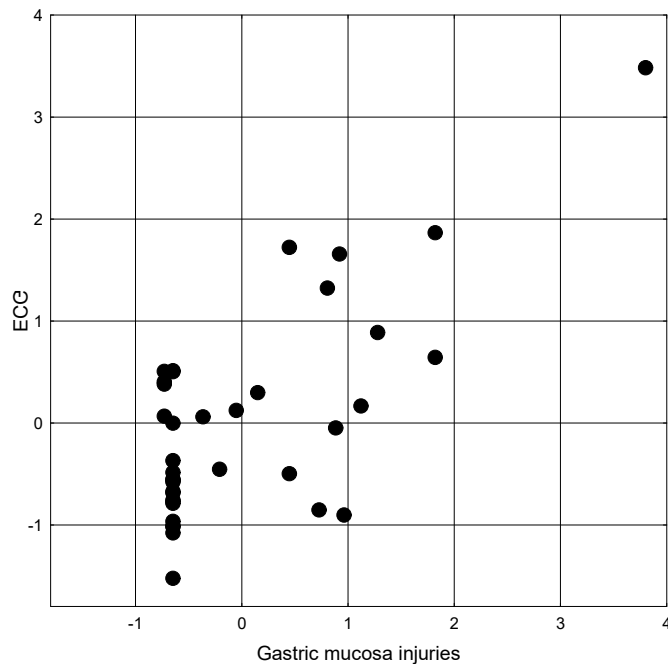
Table 2. Correlation Matrix for Gastric mucosa and EEG variables

| <i>Variables</i> | T wave ECG | S-T joint ECG | R wave ECG |
|-------------------------------|-------------------|----------------------|-------------------|
| Gastric Ulcers Amount | -0.650 | -0.500 | 0.178 |
| Gastric Ulcers Length | -0.611 | -0.497 | 0.241 |
| Gastric Mucosal Damage | -0.596 | -0.493 | 0.238 |

Note. According to the equation: $|r| = \frac{\exp[2t/(n-1.5)^{0.5}] - 1}{\exp[2t/(n-1.5)^{0.5}] + 1}$ for a sample of n=38 critical value |r| at p<0.05 (t>2.02) is **0.323**, at p<0.01 (t>2.70) is **0.420**, at p<0.001 (t>3.55) is **0.528**.

Table 3. Factor structure of Roots, which reflect post-stress damage to the gastric mucosa and myocardium

| <i>Left set</i> | R |
|--------------------------------|---------------|
| Gastric Ulcers Amount | 0.990 |
| Gastric Ulcers Length | 0.967 |
| Gastric Mucosa Injuries | 0.914 |
| <i>Right set</i> | R |
| T wave ECG | -0.913 |
| S-T joint ECG | -0.712 |
| R wave ECG | 0.282 |



R=0.706; R²=0.499; $\chi^2_{(12)}=30.2$; p=0.003; Λ Prime=0.400

Fig. 1. Scatterplot of canonical correlation between injuries of gastric mucosa (X-line) and parameters of ECG (Y-line) at intact and stressed rats

Further, it was found that such a connection is caused by the damaging effect on both targets of increasing the level of parathyroid hormone, as well as the production of aldosterone and catecholamines by enlarged adrenal glands. In addition, an increase in the level of corticosterone and sympathetic tone with a simultaneous decrease in vagal tone as well as serum calcitonin and testosterone cause damage only to the gastric mucosa, but not to the myocardium (Table 4).

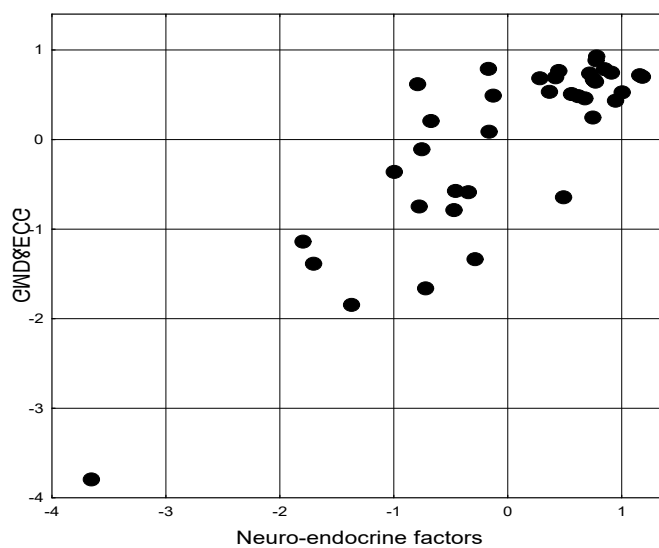
Table 4. Correlation Matrix for Neuro-endocrine and Gastric mucosa&EEG variables

| <i>Variables</i> | GU Amount | GU Length | Gastric Injuries | T wave ECG | S-T joint |
|-------------------------------------|--------------|---------------|------------------|---------------|---------------|
| Parathyroid hormone | 0.584 | 0.621 | 0.516 | -0.351 | -0.342 |
| Adrenals Mass | 0.236 | 0.384 | 0.391 | <i>ns</i> | <i>-0.192</i> |
| Aldosterone | 0.226 | 0.275 | 0.302 | <i>ns</i> | <i>ns</i> |
| 1/Mode HRV as Catecholamines | <i>0.188</i> | <i>ns</i> | <i>ns</i> | -0.356 | <i>-0.284</i> |
| AMo HRV as Sympathetic tone | 0.253 | <i>0.214</i> | 0.273 | <i>ns</i> | <i>ns</i> |
| Corticosterone | <i>ns</i> | <i>0.206</i> | 0.225 | <i>ns</i> | <i>ns</i> |
| Calcitonin | -0.229 | -0.283 | -0.251 | <i>ns</i> | <i>ns</i> |
| MxDMn HRV as Vagal tone | <i>ns</i> | -0.230 | -0.283 | <i>ns</i> | <i>ns</i> |
| Testosterone | | <i>-0.213</i> | <i>-0.204</i> | <i>ns</i> | <i>ns</i> |

Such a constellation of neuro-endocrine reactions to stressors (cold, immobilization, hunger, etc.) determines the severity of damage to the gastric mucosa and myocardium by 73% (Table 5 and Fig. 2).

Table 5. Factor structure of Roots of neuro-endocrine parameters and post-stress damage to the gastric mucosa and myocardium

| <i>Left set</i> | R |
|-------------------------------------|---------------|
| Parathyroid hormone | -0.694 |
| Adrenals mass | -0.310 |
| AMo HRV as Sympathetic tone | -0.305 |
| Aldosterone | -0.265 |
| 1/Mode HRV as Catecholamines | -0.239 |
| Corticosterone | -0.153 |
| MxDMn HRV as Vagal tone | 0.270 |
| Calcitonin | 0.258 |
| Testosterone | 0.141 |
| <i>Right set</i> | R |
| Gastric Ulcers Length | -0.969 |
| Gastric Ulcers Amount | -0.968 |
| Gastric Mucosal Damage | -0.910 |
| T wave ECG | 0.716 |
| S-T joint ECG | 0.512 |



R=0.857; R²=0.734; $\chi^2_{(45)}=72$; p=0.006; Λ Prime=0.086

Fig. 2. Scatterplot of canonical correlation between neuro-endocrine parameters (X-line) and parameters of ECG and gastric mucosa (Y-line) at intact and stressed rats

The maximum contribution (judging by the factor load on the causal neuro-endocrine root) to post-stress damage to the gastric mucosa and myocardium was precisely PTH, which was a surprise for us. After all, our search on PubMed and PMC resources revealed only one old (1989) article by Clementi et al¹⁷ that PTH injected peripherally did not interfere with the development of experimental ulcers. Conversely, when PTH was administered in cerebral ventricle the development of ulcers was significantly inhibited. The gastric secretory volume and acid output were also reduced. The possibility was discussed that this antisecretory activity of PTH may be due to a direct effect at the CNS level. However, recently Castle & Tietjens¹⁸ stated that primary hyperparathyroidism is characterised by hypercalcaemia and peptic ulcer disease in 12% patients. The debate about whether primary hyperparathyroidism increases the risk of peptic ulcer disease remains controversial in the literature. It has been shown that hypercalcaemia by any mechanism will tend to increase gastric acid production due to the role calcium plays in regulating gastrin-secreting cells. The pathophysiological mechanism is not well established, but studies point to activation of stomach calcium-sensing receptors expressed on the basolateral membrane of gastrin cells resulting in gastrin secretion, which in turn stimulates gastric parietal cells leading to increased hydrochloric acid production, explaining the association between hypercalcaemia and peptic ulcer disease.

Calcitonin, whose functional antagonism with PTH is most pronounced in relation to bone tissue, also had the opposite effect on post-stress damage to the gastric mucosa, but not to the myocardium. In our study, the gastroprotective effect of Calcitonin turned out to be weaker than expected after analyzing the literature, which in this aspect is more numerous compared to that of PTH.

Back in 1985 Clementi et al¹⁹ found that eel-Calcitonin, iv injected, decreased gastric acid secretion and inhibited the development of stress-induced ulcers in rats. In isolated rat stomach the peptide at the concentrations of 1 nM to 1 microM did not modify acetylcholine, histamine or 5-hydroxytryptamine-induced contractions. These results suggest that this peripheral activity of (Asu1,7) eel-Calcitonin does not involve a direct interference with cholinergic, histaminergic or serotonergic pathways at gastric level. At the same time Ohno et al²⁰ found that el-Calcitonin, an analogue of natural eel-Calcitonin, inhibited the development of gastric ulcers induced by WIRS (as well as by pylorus ligation, aspirin and reserpine) in rats. Similar antiulcer action was exerted by cimetidine and secretin. Moreover, once daily injections of el-Calcitonin (but not cimetidine and secretin) promoted the healing of acetic acid-induced chronic gastric ulcers not only in rats but in dogs. The healing effect persisted after the cessation of administrations. Guidobono et al²¹ later confirmed that e-Calcitonin has a high index of protection against ulcers induced by cold restraint stress or indomethacin, but not by ethanol.

Taché et al²² studied the central nervous system action of Calcitonin to influence various experimental models of gastric ulcers and gastric function in rats. Intracisternal injection of salmon Calcitonin completely suppressed gastric ulcerations induced by exposure to cold restraint stress (as well as intracisternal injection of a stable thyrotropin-releasing hormone analogue, or peroral

administration of aspirin). By contrast, intracisternal calcitonin enhanced gastric lesions elicited by peroral administration of 40% ethanol or 0.6 N HCl. Calcitonin action was dose-dependent (0.01-1 microgram) and central nervous system mediated in as much as intravenous Calcitonin, given at a dose 50-fold higher than that effective intracisternally, did not significantly modify gastric mucosal injuries elicited by aspirin or ethanol. Intracisternal injection of Calcitonin at 0.01 microgram inhibited gastric acid output by 90% in pylorus-ligated rats and suppressed gastric emptying of a liquid meal. Prostaglandin generation in the gastric mucosa was not modified by intracisternal injection of Calcitonin. These results demonstrate that intracisternal Calcitonin acts within the brain to potently prevent ulcer formation elicited by stress, thyrotropin-releasing hormone analogue, or aspirin, but is not cytoprotective against necrotizing agents. Calcitonin action is not related to modification of gastric prostaglandin generation but it may involve the inhibition of gastric secretory and motor function.

For the sake of historical justice, it should be noted that the gastroprotective effect (by pylorus ligation) of salmon calcitonin was discovered back in 1983 in the USSR²³, but remained invisible to the scientific community, probably because of the iron curtain.

After a rather long break, Calcitonin appeared as a member of its family calcitonin gene-related peptide (CGRP). CGRP is a predominant neurotransmitter from capsaicin-sensitive sensory nerves, which are widely distributed in the gastrointestinal system. The synthesis and release of CGRP is regulated by the capsaicin receptor which is known as transient receptor potential vanilloid subfamily member 1 (TRPV1). CGRP is considered a marker of afferent fibers in the upper gastrointestinal tract being almost completely depleted following treatment with the selective neurotoxin capsaicin that targets these fibers via transient receptor potential vanilloid of type-1.^{24,25}

Evangelista & Renzi²⁶ investigated the role of endogenous and exogenous CGRP in WIRS-induced gastric ulcers in rats. Authors found that WIRS produced gastric ulcers which were inversely correlated to the decrease in CGRP-like immunoreactivity observed in the whole thickness of the corpus stomach but not in its mucosal layers. Systemic administration of CGRP (100 µg/kg s.c.) produced a significant decrease in lesion index of WIRS-ulcers and this protection was inhibited by functional ablation of afferent neurons induced by capsaicin pretreatment. These findings suggest that sensory endogenous CGRP plays a defensive role in WIRS-ulcers.

The use of CGRP knockout mice has let to characterize the endogenous role of CGRP showing that the local release of this neuropeptide favours ulcer healing. Decreased levels of gastric CGRP-like immunoreactivity were observed during WIRS-ulcers (as well as acetic acid-, cysteamine-, concentrated ethanol-ulcers). Restoration of CGRP was found in animals bearing ulcers in healing status and delayed healing in mice knockout to CGRP. CGRP was able to release somatostatin from gastric D cells but its main effects on the stomach homeostasis rely on local vasodilator action during increased acid-back diffusion²⁴. In addition to increase in gastric mucosal blood flow and inhibition of gastric acid secretion, the beneficial effects of CGRP on gastric mucosa include prevention of cellular

apoptosis and oxidative injury. Therefore, the TRPV1/CGRP pathway represents a novel target for therapeutic intervention in human gastric mucosal injury²⁵.

It is well known that the secretion of Calcitonin and PTH is controlled by calcium ions. In addition, the thyroid and parathyroid glands are dually innervated by sympathetic (cervical sympathetic trunk) and parasympathetic (superior laryngeal nerve) nerve fibers. Hotta et al²⁷ found that the secretion of calcitonin (as well as T3 and T4) increased during parasympathetic nerve fibers stimulation while decreased during sympathetic stimulation. PTH secretion increased during sympathetic stimulation, but was not affected by parasympathetic stimulation.

In our study, on the contrary, a weak, but still positive correlation was found between the level of calcitonin and the HRV-marker of sympathetic tone ($r=0.27$) as well as catecholamines ($r=0.21$), but not vagal tone, in the absence of correlation with HRV parameters of PTH level (Table 6). However, the discrepancy may be due to different experimental approaches.

Table 6. Correlation matrix for neuro-endocrine factors

| Variable | Correlations | | | | | | | |
|------------|--------------|-------|-------|-------|-------|-------|-------|-------|
| | Mode | AMo | DX | TEST | CORT | Ald | PTH | CT |
| Mode | 1,00 | -0,58 | 0,39 | -0,26 | 0,18 | 0,31 | 0,05 | -0,21 |
| AMo | -0,58 | 1,00 | -0,65 | 0,21 | 0,05 | -0,27 | -0,14 | 0,27 |
| DX | 0,39 | -0,65 | 1,00 | 0,19 | -0,25 | 0,04 | -0,09 | 0,00 |
| TEST | -0,26 | 0,21 | 0,19 | 1,00 | -0,50 | -0,55 | -0,52 | 0,51 |
| CORT | 0,18 | 0,05 | -0,25 | -0,50 | 1,00 | 0,33 | 0,36 | -0,37 |
| Aldoster | 0,31 | -0,27 | 0,04 | -0,55 | 0,33 | 1,00 | 0,51 | -0,41 |
| PTH | 0,05 | -0,14 | -0,09 | -0,52 | 0,36 | 0,51 | 1,00 | -0,85 |
| Calcitonin | -0,21 | 0,27 | 0,00 | 0,51 | -0,37 | -0,41 | -0,85 | 1,00 |

It is time to move on to the analysis of the role of adrenergic and cholinergic factors in damage to the main targets of stressors.

Back in 1980, in Berger's monograph¹⁴, which reflected the results of research by employees of his laboratory (Khoma, Bolyarska, Bondarenko, Rosolovskyi) during 1971-1977, it was stated that the administration of carbacholin (10 mcg/kg) to rats 5 minutes before the administration of adrenaline (4 mg/kg), firstly, reduced mortality from pulmonary edema from 60% to 18%, secondly, reduced the index of ECG changes (on a 3-point scale) in surviving animals from 1.6 ± 0.27 to 1.1 ± 0.10 . Interestingly, a lower dose of carbacholin (5 mcg/kg) had no effect on both parameters, while a higher dose (20 mcg/kg) dramatically increased mortality to 88% (only one animal out of 8 survived). Reducing the dose of adrenaline to 1 mg/kg drastically reduced mortality (up to 10%) and, less significantly, dystrophic ECG changes (up to 1.4 ± 0.21). Preventive administration of carbacholin (20 and 40 mcg/kg) dose-independently reduced the severity of epinephrine myocardiodystrophy to 1.0 ± 0.21 and 1.0 ± 0.15 respectively. An even more noticeable cardioprotective effect of carbacholin

was found when it was administered 40-45 minutes after the administration of adrenaline (0.5 ± 0.16 and 0.6 ± 0.24 after doses of 20 and 40 mcg/kg, respectively).

Later, in the same laboratory under the leadership of Markova¹⁶, her colleagues (Koptuykh, Mysula, Khara, Deneffil) discovered that the occurrence and development of epinephrine myocardiodystrophy significantly depend on the innate resistance of rats to hypoxic hypoxia. In particular, after a dose of adrenaline of 1 mg/kg, 85÷88% of highly resistant (HR) rats and only 56÷70% of low resistant (LR) survived. Increasing the dose to 1.5 mg/kg reduced HR survival to 82% but did not affect LR survival (72%). In animals that survived, after a day, changes in ECG parameters also depended on resistance to hypoxia: in LR, the Q-T interval was lengthened by 8%, but in HR it was shortened by 5%, the T wave decreased by 11%, but in HR by 9%, with the same lowering below the isoline of the S-T junction, which was accompanied by the appearance of 122 vs 62 necrosis in the field of view of the histological preparation of the myocardium. The HRV marker of sympathetic tone in LR rats increased by 8% (from 19.0% to 20.5%), while in HR rats, the initially 14% lower sympathetic tone (16.4%) decreased by another 7%. On the other hand, in HR rats, the initially 9% higher vagal tone ($MxDMn=12$ msec vs 11 msec) increased by another 17%. The index of sympatho-vagal balance in LR rats increased from 1.73 by 19% (to 2.05) one day after administration of epinephrine, while being initially lower by 21% (1.37) in HR rats, it decreased by another 21% (up to 1.08). However, the HRV marker of circulating catecholamines (1/Mode) changed less significantly: in LR rats it increased by 2.6%, while in HR rats it decreased by 4.1%. As a result, the stress index, which includes all three HRV parameters, in LR rats increased from 74 by 22% (to 90), whereas the 20% lower stress index in LR rats decreased by another 24%: from 59 to 45.

Taken together, the presented data indicate that LR rats compared to HR are characterized by higher basal sympatho-adrenal activity and lower vagal tone, and exogenous epinephrine causes an increase in sympathetic tone and, to a lesser extent, circulating catecholamine levels and a decrease in vagal tone, i.e. acts similarly to stressors in our experiment. Instead, HR animals react to exogenous adrenaline in the opposite way: by increasing vagal tone and decreasing sympatho-adrenal activity.

Later, in the same laboratory, it was shown that the previously tested procedure (carbacholin 40 mcg/kg before adrenalin 1 mg/kg) reduced the mortality of LR rats from 30% to 12%, while HR from 12% to 0%! At the same time, in LR rats, carbacholin reduced the basal level of stress index from 57 to 36 and almost completely prevented its increase caused by adrenaline: 80 and 42 respectively. In HR rats, the lower basal level of stress index (35) carbacholin reduced even more (to 20) as well as minimized its adrenaline-induced increase from 60 to 35. Therefore, pharmacological enhancement of cholinergic effects significantly reduces the differences between LR and HR animals both at rest, and, especially, in the response of the autonomic nervous system to exogenous adrenaline as one of the main effectors of acute stress.¹⁶

Another analytical approach was applied by Zavodskaya et al.^{13,15} In these two monographs, long-term data of their laboratory were collected. It was shown that destructive changes in the myocardium

of rats developed, firstly, after the introduction of adrenaline, norepinephrine, beta-adrenoceptor agonist isadrin and indirect sympathomimetic ephedrine. Secondly, in rats and rabbits, after 0.4÷3-hour electrical stimulation of the reflexogenic zone of the aortic arch, which increases the secretion of both noradrenaline by axons of postganglionic sympathetic nerves and catecholamines (adrenaline, noradrenaline and dopamine) by cells of the medulla of the adrenal glands. It was found that prior administration of the postsynaptic alpha-adrenoblocker sympatholitin to rats completely prevented myocardial damage by exogenous adrenaline and significantly minimized the cardiotoxic effect of norepinephrine; blockade of beta-adrenoceptors with dichloroisoproterenol minimized the cardiotoxic effect of their agonist isadrine. Sympatholitin also significantly minimized damage to the rat myocardium caused by electrical stimulation of the aortic arch (which increases the impulse of the cardiac branches of the sympathetic nerve): out of 16 pretreatment animals, 11 did not show destructive changes in the myocardium, 3 had mild dystrophic phenomena, and only 2 had foci of necrosis in the left ventricle, while out of 19 control animals, severe myocardial damage developed in 15. Postsynaptic beta-adrenoblocker propranolol under similar conditions prevented the development of biochemical markers of myocardial dystrophy in rabbits: an increase in the content of lactate in the myocardium and a decrease in creatine phosphate and an increase in the activity of creatine kinase in the serum. In contrast, octadine, which blocks the release of norepinephrine by sympathetic terminals but does not block the effects of circulating catecholamines, minimized myocardial damage in rats after only 5 days of administration, whereas a single administration 40–50 min before electrical stimulation of the aortic arch was ineffective. Benzohexonium, which blocks nerve transmission in both sympathetic and parasympathetic ganglia, acted similarly. When administered once 30 min before 3-hour electrical stimulation of the aortic arch, benzohexonium had only a very weak cardioprotective effect in rats, and moreover, when administered 30 min before adrenaline injection, it even increased myocardial damage. Instead, administration of a ganglioblocker 15 min before and one hour after electrical stimulation prevented morphological damage to the myocardium in rats, and dystrophic changes in the T wave and the S-T segment of the ECG in rabbits.

Paradoxically, after completion of electrical stimulation of the aortic arch, hypothalamus, or paws of animals, the damage to the myocardium caused by it was accompanied by a 2.5-3 times *decrease* in the content of norepinephrine in it, which was maintained for the next 2 days.¹⁵

Similar to the development of myocardiodystrophy, already after 3 hours of electrical stimulation of rats, the content of norepinephrine in the gastric mucosa in 5 animals out of 11 fell below the sensitivity of the method, and in 6 it decreased by 67%; adrenaline content decreased in 10 animals, but less significantly. Similar results were obtained on another model of stress - 2-hour electrical stimulation of the negative emotogenic zone of the hypothalamus of rabbits: the content of norepinephrine in the gastric mucosa of 6 out of 8 animals fell below the sensitivity of the method, and in the remaining two it decreased by 85% and 55%, respectively (epinephrine content, detectable before stress only in 3 rabbits, after 2 hours it disappeared even in them).¹⁵

In the laboratory of Berger¹⁴, a similar effect was detected in rats after a 2-hour immobilization-cold stress: the content of norepinephrine decreased on average from 0.56 to 0.13 mcg/g, the content of adrenaline in 7 rats out of 12 was only 0.04 mcg/g, and in the remaining 5 it fell below sensitivity level of the method, while in intact animals it was 0.14 mcg/g.

Zavodskaya et al.¹⁵ concluded that with extreme irritation of the reflexogenic zone or hypothalamus, a turbulent flow of sympathetic impulses is sent to the heart and stomach, which cause the release of norepinephrine from the tissue depot in unusual quantities. Such an increased release of norepinephrine from the depot is not compensated by its resynthesis and causes the subsequent depletion of its reserves. However, the content of noradrenaline decreases in the myocardium and in the gastric mucosa (as well as in the liver, brain and aorta), also after the administration of toxic doses of noradrenaline itself as well as adrenaline or isadrin to animals. One of the mechanisms of such a phenomenon can be the inhibition of its own biosynthesis by an excess of catecholamines. However, later Meerson²⁸, confirming a decrease in the rat myocardium of norepinephrine one day after acute emotional and pain stress by 45% (as well as in the mucous membrane of the small intestine by 43%), revealed the *activation* of its biosynthesis in the atria by 33% in combination with a 40% decrease in its neuronal capture.

Berger¹⁴ provides data that 2 days after the administration of epinephrine at a dose of 3 mg/kg to rats, the content of acetylcholine in the heart decreased by a third, while the duration of its bradycardic effect did not significantly change, while a dose of 1 mg/kg probably did not affect the content of acetylcholine but continued its inhibitory effect by a third. At the same time, cholinesterase activity decreased by a quarter, and the intensity of acetylcholine bradycardia increased by 50-60% after both doses.

Markova¹⁶ later clarified that such protective cholinergic mechanisms differ in LR and HR rats. In particular, the basal content of acetylcholine in the atria of LR rats is one third higher (41.8 vs. 30 nM/g), and one day after 1 mg/kg of adrenaline it was reduced by half (to 23.4 nM/g), while in HR rats it shows only a downward trend (to 28.2 nM/g). Thus, a cardiotoxic dose of epinephrine after a day eliminated the differences between HR and LR animals with respect to atrial acetylcholine. In the ventricles, the basal levels of acetylcholine did not differ significantly (5.5 vs 5.3 nM/g), but adrenaline reduced it in LR rats to 3.3 nM/g, without affecting its level in HR rats. The total cholinesterase activity of atrial tissues in intact LR rats was lower by 8% (156 vs 170 mcM/g•h), and a day after the administration of adrenaline, the differences were completely eliminated (135 mcM/g•h each). The same difference of 8% (108 vs 118 mcM/g•h) was found for the basal activity of ventricular cholinesterase, but epinephrine reduced it in LR by 28% and in HR by 41%. The intensity of the negative chronotropic response to irritation n. vagus in HR rats significantly exceeded that in LR, and continued to increase after the administration of epinephrine, while it almost did not change in LR rats.

In another experiment by the Markova laboratory¹⁶, it was shown that in rats that died shortly after an adrenaline dose of 2.5 mg/kg, the basal stress index was 129% of the average for the sample, due to increased sympathetic tone to 119% and decreased vagal tone to 92% in the absence of deviations from the average Mode. In animals that survived, but were in a severe condition (adynamia, anorexia, etc.), the basal stress index was 88% of the average due, mainly, to reduced sympathetic tone to 91% in the absence of abnormalities in both vagal tone (101%) and a marker of circulating catecholamines (99%). At the same time, animals that satisfactorily tolerated the cardiotoxic effect of adrenaline were characterized by a slightly lower sympathetic tone (87%) in combination with increased vagal tone to 110% at the normal level of Mode, which gave a minimal level of the stress index (77%).

Therefore, our data are consistent with the data of Zavodskaya et al.^{13,15}, that myocardial damage is caused by a stressor intensification of adrenergic effects on the heart due to an increase in the level of circulating catecholamines (the lion's share of which, as is well known, is adrenaline), which is evidenced by the presence of a negative correlation between by their HRV-marker and ECG-markers of myocardial dystrophy, but not sympathetic tone, the correlation of which with the latter is absent. In contrast to Berger¹⁴ and Markova¹⁶, we did not find a cardioprotective effect of M-cholinergic influence, which is evidenced by the lack of correlation between vagal tone and ECG markers of myocardiodystrophy.

The reason for such discrepancies, in our opinion, is precisely the wide range of resistance to hypoxia in our sample. Having information and relying on previous experience^{3,5,29,30}, in our experimental design, 2 LR (76 and 80 sec) and 2 HR (285 and 317 sec) rats were tentatively included in the intact group (n=10), and such the same percentage is observed for stressed animals: 21% LR (65÷78 sec) and 18% HR (227÷294 sec). The exact opposite directionality of responses of HRV parameters in LR and HR rats both to adrenaline injection and to stressors cancels out correlations between them and ECG parameters.

Now about another important target of catecholamines and acetylcholine – the gastric mucosa.

Several stress damage models were tested in the Zavodskaya¹⁵ laboratory. It was shown that 3 days after one-hour electrical stimulation of the anterior hypothalamus, a positive reaction of feces to the presence of blood appeared in 4 out of 5 rabbits, and in the last one - on the 7th day. On the 10th day, an autopsy revealed an average of 2.6 lesions of the gastric mucosa per animal. One day after electrocoagulation of the hypothalamus, destructive damage to the gastric mucosa developed in 8 rats out of 15; the total number of tissue defects was 25, i.e. 1.7 per animal. One day after 3-hour electrical stimulation of the front paws of immobilized rats, an average of 4.6 lesions were detected. Administration of norepinephrine at a dose of 2 mg/kg caused damage in all 17 rats in the form of hemorrhagic erosions in the amount of 12.7 ± 3.3 per animal (range: 4÷27).

When clarifying the role of n. vagus, it was shown that vagotomy performed 8-25 days before one-hour electrical stimulation of the hypothalamus reduced the number of gastric mucosal lesions in 9

rabbits found the next day to 0.3 versus 2.0 in 7 rabbits that did not undergo vagotomy. In another experiment, the average number of damaged areas of the gastric mucosa one day after 3-hour electrical stimulation of the front paws of 17 immobilized rats subjected to vagotomy 2 weeks before this was 1.0 versus 3.4 in 17 animals with intact vagus nerves¹⁵. In Berger's laboratory¹⁴, vagotomy prevented the formation of ulcers in 9 out of 10 rats subjected to immobilization-cold stress.

Because during subdiaphragmatic vagotomy, not only parasympathetic, but also sympathetic nerve fibers are crossed at the same time, researchers further resorted to pharmacological analysis of the mechanisms that support and disrupt the structural and functional integrity of the gastric mucosa.

In the laboratory of Zavodskaya¹⁵, it was shown that the administration of benzohexonium to rabbits 30 minutes before the start of one-hour electrical stimulation of the hypothalamus and one hour after its completion, which achieved complete blocking of the transmission of excitation in parasympathetic and sympathetic ganglia, reduced the severity of damage to the gastric mucosa to 0.66 against 1.5 in the control. The gastroprotective effect of benzohexonium was also manifested in rats, reducing the number of damaged areas in the stomach by 5 times. However, administration of atropine sulfate at a dose of 1 mg/kg 10 min before electrification of rats, which completely eliminates the function of peripheral M-cholinergic receptors, did not protect the gastric mucosa from ulceration. On the other hand, administration of the postsynaptic alpha-adrenoblocker sympatholitin to rats one hour before electrical stimulation reduced the number of damaged areas of the gastric mucosa from 2.4 to 0.8; of beta-blocker dichloroisoproterenol - from 2.2 to 0.3; of the presynaptic sympatholytic guanethidine - from 3.3 to 2.2. The authors concluded that the efferent nerves through which impulses causing dystrophy are transmitted are adrenergic, but not cholinergic, and that the preventive effect of vagotomy is due to the crossing of precisely the sympathetic fibers in its composition.

However, there is evidence of the ineffectiveness of both alpha- and beta-adrenergic blockers, and even the aggravation of stress ulceration by beta-blockers and its attenuation by epinephrine (Berger¹⁴: review). In the laboratory of Berger¹⁴, it was shown as early as 1977 that the blockade of both alpha- and beta-adrenergic receptors with dihydroergotoxin, phentolamine or propranolol administered 30 minutes before immobilization-cold stress increased the number of ulcers. The presynaptic sympatholytic bretylium was ineffective; epinephrine (1 mg/kg) attenuated, while norepinephrine aggravated, gastric mucosal damage.

Later Esplugues et al³¹ showed that pretreatment with the beta-adrenoceptor stimulant drugs, isoprenaline or salbutamol, significantly inhibited gastric ulcers induced by stress (as well as by pylorus ligation, exogenously perfused artificial gastric juice, various iatrogenic means such as histamine, polymyxin B, reserpine and indomethacin). Long-term treatment with salbutamol accelerated the healing of experimental chronic gastric ulcer. In anaesthetized rats, salbutamol produced a dose-related increase in mucosal blood flow which may contribute to its mode of action. Authors concluded that beta-adrenoceptor agonists exert preventive and curative effects on gastric

damage induced in the rat. This effect seems specific and mediated through beta-adrenoceptor activation.

A number of authors, using a similar dose of atropine (0.8÷1.2 mg/kg), which was administered to rats 30 or 60 minutes before immobilization-cold or WIR stress, noted a decrease in the formation of ulcers (Berger¹⁴: review). Therefore, a comparative study of the gastroprotective effect of atropine at a dose of 1 mg/kg, administered 60 and 10 min before a 2-hour immobilization-cold stress, was conducted in Berger's laboratory.¹⁴ It turned out that 10 minutes is still not enough to unfold the protective effect of blockade of peripheral M-cholinergic receptors against stressors.

In other studies of the Berger laboratory¹⁴, it was shown that in rats exposed to stress, the content of acetylcholine in the gastric mucosa increased by 3.5 and 2.9 times in various experiments. This is caused by an increase in mediator release by vagal terminals in combination with a halving of acetylcholinesterase activity. Administration of both carbacholin (10 mcg/kg) and the acetylcholinesterase inhibitor ezerine immediately prior to stress increased the mean number of ulcers to 19 and 11, respectively, versus 3.5 in controls. Incidentally, let us recall the data of the laboratory of Zavodskaya¹⁵ that the introduction of the antipode of acetylcholine norepinephrine (2 mg/kg) also caused the appearance of hemorrhagic erosions in the amount of 12.7±3.3 per rat.

The ambiguity of adrenergic and cholinergic effects on the development or prevention of stress damage to the gastric mucosa is probably caused by the fact that RWIS-induced gastric mucosa damage is associated with the activation of both locus coeruleus, in which sympathoexcitatory neurones are located³², and ventrolateral periaqueductal gray, in which sympathoinhibitory neurones are located³³, as well as nucleus raphe magnus, which contains both sympathoexcitatory and sympathoinhibitory neurones³⁴.

It should also be taken into account that approximately 80% of catecholaminergic celiac ganglion neurons coexpress neuropeptide Y (NPY), and in the postganglionic sympathetic nerve fibers norepinephrine are colocalized with NPY.^{35,36}

NPY is one of the most abundantly expressed neuropeptides in the central and peripheral nervous systems and a key mediator in the responses to both acute and chronic stress. NPY occurs in the nucleus of the solitary tract and ventrolateral medulla, periaqueductal grey and locus coeruleus, hypothalamus (arcuate nucleus, paraventricular nucleus and other regions), septum, hippocampus, amygdala, basal ganglia, nucleus accumbens and cerebral cortex. Many experimental stressors induce NPY release and upregulate both NPY mRNA and its receptors' mRNA (Y₁, Y₂ and Y₅), which are responsible for the physiological actions of NPY in the periphery and brain. Acute stress upregulates NPY in the hypothalamic arcuate and paraventricular nuclei, where metabolic and stress-related signals are integrated and appropriate neuroendocrine and visceral responses are initiated. In the paraventricular nuclei, NPY activates the HPA axis and modulates the visceral stress responses mediated through corticotrophin-releasing hormone pathways.^{37,38}

NPY is involved in the emotional processing of stress. The amygdala, which is a key brain region coordinating behavioural stress responses, contains high levels of NPY and Y₁, Y₂, Y₄ and Y₅ receptors. Acute restraint stress decreases NPY expression in the amygdala. Y₁ receptor expression in the amygdala is increased by acute restraint stress. NPY is not only a stress mediator in the central nervous system but also in the periphery. The stress-related implications of NPY impact on many physiological systems including the cardiovascular system, the gastrointestinal tract, the immune system, metabolism, and adaptation to stress.^{37,38}

It is appropriate to recall that the development of behaviorally induced (stressed by mild foot shock) acute gastric lesions in rats was studied by Kristt & Freimark³⁹ back in 1973. Gastric lesions fell into two histologic classes: an acute ulcerative-hemorrhagic process with several different manifestations and a focal clear cell metaplasia of the gastric pit epithelium. The following explanation was tentatively offered to account for these findings: stress induces constriction of the blood vessels of the muscularis mucosa and results in focal mucosal infarction; the gastric pit metaplasia may reflect a response to a stress-induced impairment of the protective mucous coat.

Much later it was shown that the sympathetic constriction of splanchnic resistance vessels is co-mediated by the sympathetic triad adenosine triphosphate (ATP), noradrenaline and NPY. In addition, NPY is able to potentiate the constrictor effect of noradrenaline and ATP. Both the vasoconstrictor response to NPY and its action to augment noradrenaline- and ATP-induced mesenteric vasoconstriction are mediated by post junctional Y₁ receptors³⁸.

At the same time, stress reduces vagal tone, which is accompanied by complete inhibition of gastric juice secretion. After the termination of the stressor, the vagal tone and gastric secretion are restored to the initial level, without exceeding it.⁴⁰ But even this is enough to damage the mucous membrane with gastric juice, which has lost its resistance to it due to ischemia. It is generally agreed that luminal acid and pepsin are required for ulceration to develop. Experimental evidence suggests that backdiffusion of acid is closely related to the formation of ulcers. In the absence of overt disruption of the gastric mucosal barrier, ischaemia appears to compromise the ability of the gastric mucosa to dispose of backdiffusing acid, which then results in a decrease in intramural pH and ulceration.⁴¹ This idea was explained by the fact that the development of stress ulcers can be averted both by adrenolytics, which block adrenergic spasm of arterioles, and by cholinolytics, which block the post-stressor recovery of gastric secretion.⁴²

Incidentally, let us recall that in our rats, damage to the gastric mucosa was caused by an increase in sympathetic tone and, to a lesser extent, the level of circulating catecholamines, which is evidenced by a positive correlation between these parameters and is consistent with the cited authors. However, the negative correlation of the vagal tone with parameters of damage to the gastric mucosa indicates, contrary to the cited authors, its gastroprotective, but not gastroaltering effect under these conditions. However, this conclusion is consistent with the data of another group of authors.

Earlier experimental studies indicated that the integrity of vagal pathway was required to confer gastric protection against damaging agents. Several peptides located in the brainstem initially identified to influence vagal outflow to the stomach, as assessed by electrophysiological approach or by vagal dependent alterations of gastric secretory and motor function, were investigated for their influence in the vagal regulation of the resistance of the gastric mucosa to injury. Thyrotropin releasing hormone (TRH), or its stable TRH analog, RX-77368, injected at low doses into the cisterna magna or the dorsal motor nucleus (DMN) was the first peptide reported to protect the gastric mucosa against ethanol injury through stimulation of vagal cholinergic pathways, inducing the release of gastric prostaglandins/nitric oxide (NO) and the recruitment of efferent function of capsaicin sensitive afferent fibers containing CGRP. Activation of endogenous TRH-TRH1 receptor signaling located in the brainstem plays a role in adaptive gastric protection against damaging agents. Since then, an expanding number of peptides, namely peptide YY, CGRP, adrenomedullin, amylin, glucagon-like peptide, opioid peptides acting on μ , $\delta 1$ or $\delta 2$ receptors, nociceptin, nocistatin, ghrelin, leptin and TLQP-21, a peptide derived from VGF prohormone, have been reported to act in the brainstem to afford gastric protection against ethanol injury largely through similar peripheral effectors mechanisms than TRH. Therefore gastric prostaglandins and CGRP/NO pathways represent a common final mechanism through which brain peptides confer vagally mediated gastroprotection against injury.^{43,44}

Previous studies (review: Zhao et al⁴⁵) have demonstrated that NO can inhibit gastric acid secretion and neutrophil adhesion, improve gastric mucosal blood circulation and eliminate oxygen free radicals, thereby protecting the gastric mucosa from injury. It was reported that the expression level of iNOS increased significantly in the gastric mucosa of RWIS rats, while that of eNOS reduced significantly, indicating that the changes in iNOS and eNOS activities in the gastric mucosa are closely related to the incidence of gastric mucosal lesion (GML). NOS inhibitor can decrease the production of NO, thus exacerbating acute GML and inhibiting the healing process of chronic gastric ulcers, while NO precursor can obviously prevent the injury. It was showed that NO is involved in RWIS, and can promote the GML healing process. The mechanisms of NO in protecting gastric mucosa are as follows: (1) NO can reduce vascular permeability, inhibit platelet adhesion and aggregation in gastric mucosal vascular endothelium, and prevent thrombosis. (2) Under physiological conditions, gastric mucosal vascular endothelium synthesizes NO, which in turn regulates vascular smooth muscle tension and maintains gastric mucosa blood flow (GMBF). (3) In acute GML, NO increases GMBF by dilating the mucosal blood vessels, thus promoting gastric mucosal repair. In addition, the secretion of gastric acid can also be inhibited by NO. Upon the reaction of stimulus against gastric mucosa, enterochromaffin cells and mastocytes can release histamine to stimulate parietal cells for gastric acid production, thus aggravating the mucosal lesion. In addition, endogenous NO can inhibit the stimulation of histamine through parietal cells, thus reducing gastric acid secretion and protecting gastric mucosa. It has been found that, through *in vivo* and *in vitro* experiments, the NO

donor FK409 and sodium nitroprusside can markedly suppress the gastrin-induced increase in histamine release and gastric acid secretion in rats, and NOS inhibitor further increases gastric acid secretion. Gastric mucous cells promote NO synthesis by expressing high-level NOS, and enhance the mucous barrier through the NO effects of promoting mucin synthesis and secretion. Based on the findings of previous experiments, RWIS-induced GMLs can weaken the synthesis and secretion of gastric mucus by reducing nNOS activity, while the NO donor can increase nNOS activity and mucus secretion.

More recently, on the example of nerve terminal innervating cerebral arteries at the base of the brain, has been shown that NO, which is not stored in vesicles, is colocalized and co-released with ACh, which is stored in the vesicles. NO is synthesized from L-arginine in the presence of NOS. The neuronal NO plays a major role in cerebral neurogenic vasodilation, which is mediated by activation of guanylate cyclase and cGMP synthesis in the smooth muscle cell. Electrical stimulation of a central cholinergic system originating in the nucleus basalis of Meynert and substantia innominata has been shown to contribute to the cortical vasodilator response via activation of muscarinic cholinergic receptors, although nicotinic cholinergic receptors have been shown to consistently mediate vasodilator response in both cortical circulation and large arteries at the base of the brain. NE released from the sympathetic nerve, acting on presynaptic β_2 -adrenoceptors located on the neighbouring parasympathetic nitrenergic nerves, however, facilitates NO release with enhanced vasodilation. This axo-axonal interaction mediating NE transmission is supported by close apposition between sympathetic and parasympathetic nerve terminals, and has been shown in vivo at the base of the brain and the cortical cerebral circulation. This result reveals the physiological need for increased regional cerebral blood flow in 'fight-or-flight response' during acute stress. Furthermore, nicotinic ACh receptors on sympathetic nerve terminals mediate release of NE, leading to cerebral nitrenergic vasodilation.^{46,47}

If we assume that similar processes take place with the participation of vagal efferents and blood vessels of the gastric mucosa, which is quite likely, then the gastroprotective effect of vagal innervation can be associated with the recruitment of gastric prostaglandins-CGRP-NO mechanisms.

Zhao et al⁴⁵ give arguments, that the role of the vagal nerve is likely to be dual, as it can mediate both mucosal damaging and protective effects. RWIS-induced gastric dysfunction is mainly caused by the enhanced parasympathetic activity. In other words, there is a neural circuit ("medullary gastrointestinal center-gastrointestinal wall plexus loop") between the medulla oblongata and the gastrointestinal tract. Under RWIS, the information of gastrointestinal motility is transmitted as follows: Information \rightarrow vagal afferent nerves \rightarrow NTS \rightarrow DMV/NA, while those of medullary efferents are disseminated as follows: DMV/NA \rightarrow vagal efferent nerves \rightarrow gastrointestinal wall plexuses, thereby causing gastric hyperkinesia, increasing gastric acid secretion and reducing gastric mucus secretion, and ultimately leads to GML and fecal impaction. However, in the case of electrical stimulation of NTS in normal rats, gastric motility may be inhibited, and a possible reason for this is

that excitement of the NTS activates inhibitory neurons in the DMV, thus suppressing gastric motility through a non-cholinergic neural pathway. In addition, gastric motility was significantly inhibited when electrical or chemical stimulation induced neuronal excitation in the NA and DMV, indicating that excitation of the NA and DMV also exerts an inhibitory effect on gastric motility. This is probably due to the fact that the activity of the higher center (*e.g.*, anterior hypothalamus) eliminates the inhibition of medullary visceral centers on the stomach during RWIS, thereby causing gastric hyperkinesia and increasing gastric acid secretion.

Despite the fact that the hypothalamic-pituitary-adrenocortical (HPA) axis is the cornerstone of the general adaptation syndrome and the famous triad^{1,2}, the role of its hormones in the pathogenesis of stress injuries of the gastric mucosa and myocardium is still a matter of debate.

There are two opposite points of view regarding the influence of stress-induced activation of HPA system on the stomach. According to the widely held view, glucocorticoids released during stress are ulcerogenic hormones and, therefore, stress-induced activation of HPA system is harmful. Some studies have found that RWIS leads to the elevation of blood corticosterone and adrenocorticotrophic hormone levels in rats, and their levels in plasma also gradually rise over a prolonged period of stress. This seems to indicate that the activity of the HPA axis is enhanced during RWIS. However, removing the pituitary glands and adrenal glands or administering phenoxybenzamine (adrenergic α -receptor blocker) has little impact on RWIS-induced GML, gastric hyperkinesia and RWIS-induced gastric acid secretion, but severing the subphrenic vagus nerves or consuming atropine can significantly alleviate and even cure RWIS-induced GML. This suggests that the HPA axis does not play a major role in RWIS-induced GML, and the peripheral nervous mechanism of RWIS-induced GML is mainly through the enhanced parasympathetic activity. Therefore, according to Zhao et al⁴⁵, the nervous mechanism of RWIS-induced gastrointestinal dysfunction in rats is mainly the "enhanced activity of parasympathetic nervous system", rather than the traditional ideas of the "enhanced activity of sympathetic-adrenal medulla system" and "HPA axis". However, we cannot unconditionally agree with this in view of the concept of vagally mediated gastroprotection against injury^{43,44} and our own data in this regard.

However, in our study, we found only a minimal factor load on the neuro-endocrine canonical root from the side of corticosterone, which also indicates the insignificance of its causal influence on post-stress damage to the gastric mucosa and myocardium. This is only partially consistent with the old data of the Berger's laboratory¹⁴ that the introduction of hydrocortisone (2 mg/200g) for 2 days before immobilization-cold stress increases the number of ulcers from 3.6 ± 0.5 to 7.7 ± 1.9 , and the data of one of the latest studies⁴⁸ that phytoadaptogen (Banhasasim-tang) positively ameliorated in rats cold restraint stress-induced gastric hemorrhage with decrease in serum stress-related biomarkers such as ACTH and corticosterone (as well as epinephrine and dopamine). However, this is perfectly consistent with the dual role of corticosterone, similar to n. vagus, as both a gastroaltering^{1,7,14,15,28,42,48} and a gastroprotective^{15,49,50,51,52,53,54} factor.

It is interesting that the initiator and apologist of the last concept are two laboratories located in St. Petersburg. The results of their investigations are opposite to traditional view. Back in 1981, in the Zavodskaya¹⁵ laboratory, it was shown that adrenalectomy worsens damage to the gastric mucosa of rats caused by electrocoagulation of the anterior or posterior hypothalamus. Thus, among 15 control rats, destructive damage to the gastric mucosa developed in 8, an average of 1.7 per animal, whereas among 15 previously (20-30 days) adrenalectomized rats, 13 developed, an average of 3.0 per animal. Starting from 1998 in the Filaretova⁴⁹ laboratory, research in this direction was continued at a higher methodological level. To estimate the action of glucocorticoids released during stress on the gastric mucosa, the effects of glucocorticoid deficiency or occupation of glucocorticoid receptors by the antagonist RU-38486 on the formation of stress-induced gastric erosions were estimated. The reduction of stress-induced corticosterone release (induced by various experimental approaches) markedly potentiated a gastric erosion formation caused by stress and acute corticosterone replacement, mimicking stress-induced corticosterone response, prevented this erosion-potentiating effect. The administration of RU-38486 also caused a significant increase of vulnerability of gastric mucosa to stress action. Corticosterone replacement which mimics the corticosterone rise significantly reduced erosion areas of gastric mucosa in adrenalectomized rats. Thus, an acute stress-induced increase of glucocorticoids has a gastroprotective action against stress-induced gastric injury. Authors also showed that various ulcerogenic stimuli, similar to stress, induce an increase in glucocorticoid production that in turn helps the gastric mucosa to resist against a harmful action of ulcerogenic stimuli. Gastroprotective action of glucocorticoids may be mediated by multiple actions, including maintenance of glucose homeostasis, gastric mucosal blood flow, mucus production and attenuation of enhanced gastric motility and microvascular permeability. For maintenance of gastric mucosal integrity glucocorticoids may cooperate with prostaglandins. Furthermore, glucocorticoids exert a compensatory gastroprotective role in the case of impaired gastroprotective mechanisms provided by PGs, NO, and capsaicin-sensitive sensory neurons. The authors still admit that after single administration of glucocorticoids, there can arise gastroprotective and ulcerogenic effects. The initial gastroprotective effect that glucocorticoid hormones have, even after their single administration can be transformed into an ulcerogenic effect with a prolongation of the hormonal action, but not of the hormone dose.

Since Selye⁵⁵, mineralocorticoids have been considered glucocorticoid antagonists. In the laboratory of Berger¹⁴, administration of deoxycorticosterone acetate (DOCA, 1 mg/200g) for 4 days before immobilization-cold stress, as opposed to hydrocortisone, reduced the number of ulcers from 3.6 ± 0.5 to 1.6 ± 0.8 . This is consistent with the data obtained in the same period, that aldosterone exhibited antiulcer actions in fasted rats stressed by the forced exertion technique, but only when multiple subcutaneous injections were made. The antiulcer actions of aldosterone (and DOCA) were not mediated via an inhibitory effect on gastric secretion.^{56,57} Interestingly, the main object of these studies was carbenoxolone – a semisynthetic succinyl ester of glycyrrhizinic acid (licorice root

substance) which has been used as an effective treatment for peptic ulceration since the 1960s.⁵⁸ Carbenoxolone given subcutaneously did not inhibit ulcer formation while intragastric administration of carbenoxolone significantly inhibited it.⁵⁶ Authors concluded that the beneficial antiulcerogenic action of carbenoxolone is due to a direct effect on gastric mucosa and is not related to an aldosterone-like component. Koo et al⁵⁹ shown that intragastric administration of carbenoxolone, given 30 min before restrain cold stress, exhibited similar actions as verapamil - significantly prevented stress-induced mucus depletion and gastric ulceration.

Stewart et al⁶⁰ have been evaluated the effect of the carbenoxolone on enzyme complex 11-beta-hydroxysteroid dehydrogenase that consisting of 11 beta-dehydrogenase and 11-oxoreductase responsible for the interconversion of cortisol to cortisone in man. It is known that inhibition of 11 beta-dehydrogenase results in cortisol acting as a potent mineralocorticoid. Authors shown that carbenoxolone given to six volunteers in metabolic balance produced sodium retention with suppression of the renin-angiotensin-aldosterone system. Plasma potassium fell, although there was no kaliuresis. This was associated with inhibition of 11 beta-dehydrogenase (as measured by a rise in the plasma half-life of [11 alpha-³H]cortisol). Thus, the mineralocorticoid activity of carbenoxolone is mediated via cortisol by inhibition of 11 beta-dehydrogenase. Carbenoxolone, however, also inhibited 11-oxoreductase activity (as measured by the generation of cortisol after oral cortisone acetate), and this may relate to its effect on renal potassium excretion.

Paradoxically, that medical use of carbenoxolone is limited just by side effects of a mineralocorticoid aldosterone-like property with hypokalemia, weight gain, hypertension, and retention of sodium, chloride, and water.⁶¹

Suleyman et al⁶² showed that nimesulide, a non-steroidal, anti-inflammatory drug, is gastro-protective in intact rats, but produces ulcerogenic effects in adrenalectomized rats. The objective of their study was to determine whether adrenal gland hormones are involved in the anti-ulcer effects of nimesulide. The results revealed that nimesulide produces gastric ulceration in adrenalectomized rats, which is prevented by prednisolone and adrenaline, while DOCA did not cause any gastroprotective effect: the mean ulcer area was 17.2 mm² in the DOCA-treated nimesulide group and 18.0 mm² in control.

However, in our study, contrary to the cited ones, aldosterone was neither a gastroprotective nor a neutral factor, but on the contrary, judging by the factor load on the neuro-endocrine root, it exerted an ulcerogenic effect, even somewhat stronger than corticosterone. At the same time, the mass of the adrenal glands gave more factor load than each of their hormones separately. It would be tempting to explain this by an additional effect of medulla catecholamines.

Our conclusion about the ulcerogenic effect of aldosterone is supported by Pawlik's et al⁶³ data that the inhibition of angiotensin-converting enzyme or the blockade of angiotensin AT-1 receptors, that is, eliminating the effect of aldosterone, affords protection against acute gastric mucosal injury in rats.

Despite the expectations based on the classical position of Selye⁶⁴, that the administration of both glucocorticoids and mineralocorticoids to animals increases the cardiotoxic effect of catecholamines, in our study no correlation was found between the serum content of these hormones and ECG-markers of myocardial damage. We explain this situation by the peculiarities of the timing of post-stress corticosteronemia, beautifully illustrated by Meerson.^{30,42} It was found that an hour after the start of a 6-hour emotional and painful stress, the level of corticosterone exceeded the initial level by 280%, immediately after its end - by 250%, after another 2 hours – by 225%, and the next day (30 hours after the onset of stress) – by only 30%, which practically coincides with our data (+15%) and gives reason to assume that the dynamics of corticosteronemia were approximately the same during and after 4-hour WRIS in our experiment. And so, corticosterone (as well as aldosterone) is still involved in stress damage to the myocardium, as it has been shown in relation to the gastric mucosa.

Interestingly, 42 hours after the onset of emotional and painful stress, the level of corticosteronemia rose again to 180% of the initial level.^{30,42} And in the experiments of the Berger's laboratory¹⁴, the ECG was recorded exactly on the 2nd-3rd day after the injection of adrenaline (1.5 mg/kg). It was found that the previous (2 hours) administration of both hydrocortisone (2.5 mg/kg) and DOCA (2.5 mg/kg) did not significantly affect ECG markers of myocardial dystrophy (1.4 ± 0.14 and 1.5 ± 0.14 respectively vs 1.3 ± 0.10). This is consistent with our data on the lack of correlation of post-stress levels of both corticosterone and aldosterone with ECG markers of myocardiodystrophy. At the same time, it should be noted that a 5-fold increase in the dose of corticosteroids when administered for 2 days still significantly aggravated epinephrine myocardiodystrophy (ECG change index 1.7 ± 0.14 for both corticosteroids). One of the mechanisms of such an aggravating effect should be considered a decrease in the content of acetylcholine in the heart by 30-33%.¹⁴

Testosterone, judging by the results of the correlation analysis (Table 4), turned out to be a weak gastroprotective factor, as well as calcitonin, with which it is positively related, in contrast to negative relationships with gastroaltering factors such as PTH, aldosterone and corticosterone, but not catecholamines (Table 6).

Our conclusion contradicts the data of the Zavodskaya laboratory¹⁵ about twice the number of destructive damage to the gastric mucosa caused by electrical stimulation of the paws of immobilized rats in males (6 ± 2 vs 3 ± 1), whose testosterone level is an order of magnitude higher than in females. Castration of animals 12-19 days before stressor exposure did not cause destruction by itself, but increased their number in males to 13 ± 4 , and in females to 9 ± 4 , that is, it also minimized sexual dimorphism in the resistance of the gastric mucosa to stressors. However, administration of testosterone propionate to uncastrated males and diethylstilbestrol propionate to females also resulted in aggravation of gastric stress injuries. The introduction of chorionic gonadotropin, which stimulates the release of one's own sex hormones, also increased the number of post-stress destructions in uncastrated males and females. The researchers came to the conclusion that both depriving the body of

sex hormones and saturating it with synthetic analogues lead to a decrease in the resistance of the gastric mucosa to the pathogenic influence of stressors.

We were not lucky enough to find more publications on the influence of testosterone on the development of stress-induced damage to the gastric mucosa, so we are forced to limit ourselves to the discussion of other models with the caveat that their pathogenesis is different from the stressor to one degree or another.

Rao & Saifi⁶⁵ in pylorus ligated male rats discovered that testosterone and cimetidine when used alone protected from ulceration while when used in combination the degree of protection was decreased. Castration per se had no effect on ulcer index but potentiated cimetidine induced gastric ulcer protection.

László et al⁶⁶ found that macroscopic mucosal damage and microvascular serum albumin leakage developed in the stomach of male rats 24 h after the administration of cysteamine. This mucosal injury was prevented by orchidectomy and by the pretreatment with the antiandrogen cyproterone. It was also shown that pretreatment with testosterone dose-dependently aggravated cysteamine-induced mucosal injury.

While Loginov and al⁶⁷ concluded that in gastric ulcer patients there were low concentrations of testosterone, hydrocortisone, estradiol, and progesterone.

Despite the fact that female sex hormones were not determined in our study, it is very appropriate to discuss their participation in the pathogenesis of gastric ulcer.

The female sex hormones (progesterone, estrogen and a combination of both) were found to have significant antiulcer activity in almost all the models (stress-, drug-, pylorus ligation induced). However, they did not affect the acidity or volume of gastric secretion in Shay's pyloric ligation model. As a result their antiulcer activity could not be explained by the effects on gastric acidity but by effects on other factors which may include enhanced mucus activity, or increase in parietal cells activity and maintenance of mucus integrity.⁶⁸

A more detailed study was conducted by Kurt et al.⁶⁹ To investigate the protective effects of estrogen and progesterone administrations on gastric mucosal barrier of rats applied ovariectomy, cold and immobility stress. It is shown that the levels of mucus and phospholipids were decreased in the rats applied ovariectomy and stress as compared to the control groups ($p < 0.001$). The increase determined the mucus and phospholipids levels in estrogen and progesterone administered rats as compared to stress applied group ($p < 0.001$). While the cold and immobility stress causes important damages in gastric mucosa, estrogen and progesterone administrations has protective effects in ovariectomized rats. Authors concluded that the estrogen and progesterone administration prevents the stress that caused decrease in the levels of mucus and phospholipids, thus females are more resistant to gastric ulcer rather than males due to their sex hormones.

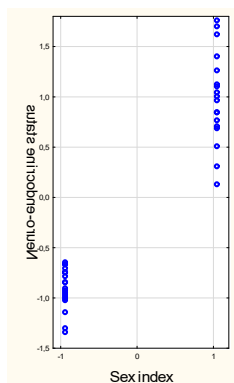
We were able to find only one study regarding the effect of testosterone on myocardial stress injury. Ribeiro et al⁷⁰ showed that papillary muscle contractility was preserved in the orchidectomized

rats after myocardial infarction and was reduced when testosterone was replaced. Their results support the view that testosterone deficiency prevents myocardial infarction contractility dysfunction.

If the male is evaluated with a conditional one point, and the female with two, then it is possible to operate with the sex index, which was found in the factor structure of the neuro-endocrine canonical root. The sex index is most negatively correlated with the serum level of testosterone ($r=-0.89$), significantly stronger with calcitonin ($r=-0.63$) and on the verge of significance with sympathetic tone ($r=-0.31$). Instead, the sex index is positively correlated with the mass of the adrenal glands ($r=0.80$) and serum levels of corticoids such as aldosterone ($r=0.63$) and corticosterone ($r=0.47$), as well as PTH ($r=0.61$). This is explained by significantly higher levels of PTH (in our sample by 20%), corticosterone (by 37%) and, to a lesser extent, aldosterone (by 9%) in females than in males, as well as undoubtedly not recorded in this study estradiol and progesterone, instead drastically lower levels of testosterone and, to a lesser extent, calcitonin (by 40%) and sympathetic tone (by 20%).

At the same time, the role of other sex-linked neuro-endocrine factors (gonadotropic hormones and their releasing factors, etc.) and, in particular, the recently discovered sexual dimorphism of a number of EEG parameters in humans is quite likely.^{71,72}

But even such a limited set of neuro-endocrine parameters allows you to recognize the sex of a rat without examining the genitals with an accuracy of 88% (Fig. 3).



$R=0.938$; $R^2=0.880$; $\chi^2_{(7)}=71$; $p<10^{-6}$; Λ Prime=0.111

Fig. 3. Scatterplot of canonical correlation between Sex index (X-line) and neuro-endocrine parameters (Y-line) at intact and stressed rats

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Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

The carry out of experiments was approved by the Ethics Committee of the Ukrainian Scientific Research Institute of Medicine of Transport (protocol No35; 05.10.2022).

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