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THE INFLUENCE OF MELANIN ISOLATED FROM ANTARCTIC YEASTS ON CORTISOL BLOOD LEVEL OF RATS IN CONDITIONS OF STRESS ACTION

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The influence of melanin on cortisol blood level in the rats in conditions of stress action. T.M. Falalyeyeva, O.I. Tsyryuk, N.V. Chyzhanskaya, V.P. Zharova

Abstract. Immobilizational stress, exposure time of which was 24 hour evoked in gastric mucosa of rats formation of ulcers, erosions and hemorrhages with simultaneous drastic increase of cortisol blood level. Melanin defended gastric mucosa against development of injuries evoked by stress and at the same time reduced cortisol blood level by 1,81 times. It was concluded that a decrease in cortisol blood level against a background of melanin action is the result of active influence of melanin on one of the links of mechanism of cortisol liberation.

Key words: melanin, stress, gastric mucosa, injury, cortisol

1. Introduction

Although today enough effective pharmaceutical substances were developed and they widely are used for treatment of ulcer disease of stomach and duodenum, amount of drugs for prevention of development and recurrence of ulcer disease is limited. Considering that stress, which continually accompanies people in present society, is starting device of mechanism of ulcer disease pathogenesis, efforts of many scientists are devoted to study of mechanism action of stress on gastric and duodenal mucosa and generation of prophylactic drugs from stress action on it. It is known that stress activates sympathoadrenal system, which leads to increase of blood level of stress hormone cortisol [12,16].

It is proved participation of cortisol in formation of gastric ulcer of different genesis [2,4,11,14,17] including ulcers evoked by stress [6,7,13]. Analysis of modern literature and our previous data allow to state that natural drugs on the basis of polyphenol compounds are possible perspective agents for prophylaxis of ulcerous injuries of gastric and duodenal mucosa. Melanin which is the product of life activity of yeast-like fungi *Nadsoniella nigra sp. X-1* belongs to these drugs.

In connection with this the aim of the study was to investigate the influence of melanin on cortisol blood level in the rats in conditions of stress action .

2. Object and methods of investigations

The investigations were done on male white pubertal rats. Formulation of the experiments was in accordance with Convention on bioethics of Council of Europe (1997), Helsinki declaration of World Medical Association (1996), European convention about vertebrates protection, which are used for experimental and others scientific aims (Strasbourg, 1985), general ethical principles of experiments on animals, accepted by the 1st National Congress of Ukraine on bioethics (September of

2001 year), others international agreements and national legislation in this field [1,5,8,9].

The animals were divided into three groups for 6 in each. The animals were starved for 24 h prior to experiments, but were allowed free access to water. The 1st group was intact control. Blood sampling were done in 24 hours of starvation. The rats of 2nd and 3rd groups were done to the action of "social" stress (immobilizational stress in modification by Groisman and Karevina [3]). Before beginning of stress action to the rats of 2nd group we injected 0,9 ml of saline per os. To the rats of 3rd group we injected melanin in dose 5 mg/kg per os, dissolved in 0,9 ml of saline. In 24 hours of stress action we carried out sacrifice and get the blood for cortisol level determination. Then we got stomach, cut its along lesser curvature, turned out, well washed by phosphate buffer PBS at 4°C.

Melanin which we used in our experiments is the product of life activity of yeast-like fungi *Nadsoniella nigra sp. X-1*. Microorganisms were seed from samples of vertical rocks of island Galindez (Academic Vernadskyy Ukrainian Antarctic station). Melanin from present origin consists of polyphenolcarboxylic complex by 95%.

Concentration of cortisol we determined by method of solid-phase immunoassay in the blood serum of intact and experimental animals by use assay kit "SteroidEIA-cortisol". Using circadian rhythm of cortisol release in the blood [18], we always begun and ended experiments at the same time. Thus blood sampling for testing of cortisol blood level we did at the same time of day.

All results are performed as Mean±SD by using Students T-test.

3. Results and discussion

In results of our investigations it was established that in rats of control group the injuries in gastric mucosa were absent. In 24 hours of stress action ulcers, erosions and massive hemorrhages developed in gastric mucosa (Table. 1). Injection of melanin to the rats before beginning of stress action completely removed ulcers formation, amount of erosions and massive hemorrhages per one stomach decreased by 78,3% and 67,9% respectively.

Table 1

The influence of melanin on injuries in gastric mucosa of rats evoked by immobilizational stress

| Injuries | Amount of injuries per one stomach, (M±SD) | | |
|---------------------|--|--|--|
| | control | stress + saline (0,9 ml, per os) (n=10) | stress + melanin (5 mg/kg, per os) (n=10) |
| Ulcers | 0 | 1,03±0,56 | 0*** |
| Erosions | 0 | 4,48±0,51 | 0,97±0,62** |
| Massive hemorrhages | 0 | 1,56±0,76 | 0,50±0,34** |

Note: ** - p<0,01, *** - p<0,001 versus stress; n – amount of animals.

In control group of rats concentration of cortisol in the blood serum was $20,46 \pm 0,58$ nmol/l (Table.2). In rats of 2nd group in 24 hours of stress action concentration of cortisol strongly increased and was $105,89 \pm 2,65$ nmol/l. It was more by 417,5% against control. In rats of 3rd group, which before stress action were injected melanin, concentration of cortisol in the blood serum after finish of stress action was $67,66 \pm 3,25$ nmol/l. It was more by 230,7% against control but in 1,81 less than in rats of 2nd group, which before stress action were treated by saline.

Table 2

The influence of melanin on concentration of cortisol in the blood serum, $M \pm SD$

| The groups of animals | n | The concentration of cortisol in blood plasma (nmol/l) | Effect in percent versus control |
|----------------------------------|---|--|----------------------------------|
| Control | 6 | $20,46 \pm 1,42$ | |
| Stress + saline (0,9 ml, per os) | 6 | $105,89 \pm 6,49^{***}$ | > 417,5% |
| Stress+melanin (5 mg/kg, per os) | 6 | $67,66 \pm 7,96^{***}/##$ | > 230,7% |

Note: *** - $p < 0,001$ versus control, ## - $p < 0,01$ versus the group stress + saline, n – amount of animals.

As opposed to Lechin et al. [15], which were showed that cortisol blood level in patients with ulcer disease of duodenum strongly increases, but after healing of ulcer come back to control values, in our experiments melanin in 1,81 times diminished cortisol blood level which enhanced by stress influence in 5,18 times. Taking into account that melanin by 100% protected gastric mucosa from ulcer formation and essentially decreased formation of erosions and massive hemorrhages we concluded that decrease of cortisol blood level evoked by melanin is the result of active influence of melanin on one of the links of mechanism of cortisol release.

This mechanism is that some physiological or exogenous stress influence on organism leads to stimulation of corticotrophin-releasing-hormone release, which is secreted mainly by paraventricular nuclei of hypothalamus and is basic regulator of synthesis and secretion of adrenocorticotrophic hormone in adenohipophysis. The last stimulates the release of glucocorticoides including cortisol from adrenal cortex which ensure the adaptation of organism to stress agents of environment. Stress agents of environment includes all influences on organism: from infectious and traumatic agents to emotional stress.

For some time past it was shown that glucocorticoides in supraphysiological doses protect gastric mucosa from powerful ulcerogenic influences [10] through mechanism of improvement of microcirculation in gastric mucosa. Thus smaller but unreduced to the control cortisol blood level is important factor which protects gastric mucosa from stress injuries.

4. Conclusion

Immobilizational stress exposure time of which was 24 hour evoked in gastric mucosa of rats formation of ulcers, erosions and hemorrhages with simultaneous drastic increase of cortisol blood level. Melanin protected gastric mucosa from development of injuries evoked by stress and at the same time reduced cortisol blood level by 1,81 times. Abrupt decrease of cortisol blood level evoked by melanin is the result of active influence of melanin on one of the links of mechanism of cortisol release.

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