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Induction of metallothioneins by ethanol and morphine

Metallothioneins (MT) are the widespread proteins in the animal world. These proteins are characterized by low invariability of their structure. Although isolated from different organs of different animals they show slight differences in the aminoacid composition between one another. The number of aminoacids is fixed in every animal group, that is 60 (or 61) aminoacids, 20 of which are the cysteins radicals, which makes over 30% of the aminoacid composition. Such a big amount of cysteins which include the reactive sulfhydryl groups -SH determines the metallothionein's functions (1,2,4).

Metallothioneins take part in homeostasis of the ions of the metals which are necessary for proper metabolism of the organism (zinc, copper), biosynthesis regulation and zincproteins activity (for example the activity of the zinc-dependent transcription factors). They also take part in the detoxication of the tissue from toxic metals. Apart from these, they also protect the tissue from the oxygen-free radicals, radiation, electrophilic pharmacologic agents used in the cancer therapy, and the mutagens (5,7,9,11,14).

There are two groups of MT proteins: MT-I and MT-II coded by different genes. The other two groups of MT-proteins: MT-III and MT-IV were isolated from the brain (MT-III) and the stratified epithelium (MT-IV) (13).

The metallothionein's synthesis induction is influenced by many factors: heavy metals, inflammatory factors, free radicals, glycocorticoids and the pharmacologic agents (8,14).

The aim of this work was to indicate the metallothionein's level in rat tissues intoxicated by ethanol and morphine. The experiment was to state if the narcotic agents cause the change in the metallothionein's level in the liver, brain and kidneys of the intoxicated animals.

MATERIAL AND METHODS

MATERIALS

The experiments were conducted on the 6-month-old Wistar rats, 150-200 g of weight each, which were divided into 3 groups. The first group was on the normal diet (LSM dry food and the redistilled drinking water) and that was the control group. The second group was given intragastrically 20% solution of ethanol for 5 days in the dose of 2 g/kg of the body weight. The third group was given

morphine intraperitoneally (15 mg/kg b.w. — on the first day, 30 mg/kg b.w. — on the second day, 45 mg/kg b.w. - in the third day, 60 mg/kg b.w. — on the fourth day and 50 mg/kg b.w. — on the fifth day). After 5 days the animals were anaesthetized with kethamine and their tissues were taken for further examinations.

LEVEL OF METALLOTHIONEIN

The level of metallothioneins was determined by the cadmium-hemoglobin affinity assay (3), using the cadmium isotope from Du Pont.

Tissues were prepared for MT analysis by homogenization in 4 vol. of 10 mM Tris-HCl. The homogenate was centrifuged at 10,000 g for 10 min, and the supernatant fraction was heated for 2 min in a boiling water bath. The heated samples were then centrifuged at 10,000 g for 2 min to remove precipitated proteins.

A 200-µl aliquot of each heat-denaturated tissue supernatant was placed in a 1.5-µl polyethylene microcentrifuge tube. Carrier-free ^{109}Cd (Du Pont) was combined with $CdCl_2$ in 10 mM Tris-HCl buffer, pH 7.4, to yield a Cd concentration of 2.0 mg Cd/µl and radioactivity of 1.0 mCi/µl. 200 ml of the ^{109}Cd solution were then added to each centrifuge tube, and allowed to incubate with each sample for 10 min. Then 100 µl of a 2% bovine hemoglobin solution (Sigma) were added to the tubes and mixed. The tubes were heated in a 100° C boiling water bath for 2 min and another 100 µl of the 2% hemoglobin solution was again added, and the heating, cooling, and centrifuging were repeated. This procedure resulted in a tightly packed pellet and a clear supernatant fluid from which a 500-µl aliquot was transferred to a gamma counting tube. The amount of radioactivity in the supernatant fraction was then measured on Beckman counter. Blank samples with buffer in place of the MT sample and samples to determine total activity (buffer in place hemoglobin) were run with each assay.

The results were analyzed statistically with the Wilcoxon test accepting the differences as intrinsic at the intrinsicity level $p < 0.05$. The results obtained were presented in the table.

Table 1. The level of metallothioneins (in µg/g tissue) in liver, brain and kidneys of rats intoxicated with ethanol and morphine

	Mean and standard deviation	Range	Significance level
		Liver	
Control	2.47 ± 0.36	(1.79 - 3.15)	
Ethanol	9.34 ± 1.70	(6.89 - 11.79)	$p < 0.0001$
Morphine	7.96 ± 1.63	(5.28 - 10.64)	$p < 0.0001$
		Brain	
Control	0.72 ± 0.15	(0.57 - 0.97)	
Ethanol	7.30 ± 1.52	(5.14 - 9.46)	$p < 0.0001$
Morphine	11.1 ± 1.75	(8.00 - 14.20)	$p < 0.0001$
		Kidneys	
Control	4.13 ± 0.90	(2.68 - 5.58)	
Ethanol	6.63 ± 1.54	(4.25 - 9.06)	$p < 0.001$
Morphine	8.11 ± 2.13	(4.89 - 11.33)	$p < 0.001$

RESULTS

Table 1 shows the metallothionein's level in rats' tissues intoxicated by ethanol and morphine.

As the numerical data presented in the table show the level of metallothioneins in tissues of the control group is differentiated. The highest level of metallothioneins is ascertained in kidneys and the liver (4.13 +/– 0.90 μg/g of tissue and 2.47 +/– 0.36 μg/g respectively). The considerably lower level of metallothioneins was ascertained in the brain (0.72 +/– 0.15 μg/g).

In the group of ethanol intoxicated animals the level of the metallothioneins was 9.34 +/– 1.70 μg/g — in the liver, 7.30 +/– 1.52 μg/g — in brain and 6.63 +/– 1.54 μg/g tissue — in kidneys.

In the group of the morphine intoxicated animals the level of metallothioneins was 7.96 +/– 1.63 μg/g — in the liver, 11.10 +/– 1.75 μg/g — in the brain and 8.11 +/– 2.13 μg/g tissue — in kidneys.

DISCUSSION

In the control group of animals the highest level of metallothioneins was ascertained in the liver and kidneys which manifests the role of these tissues in the microelements homeostasis, in the possibilities of the metallothionein's synthesis and the detoxication of tissue processes.

In the groups of the intoxicated animals both ethanol and morphine cause the increase in the MT level in all the examined tissues while the differences in the strength of the MT induction according to a tissue are well seen. Ethanol and morphine induce the MT synthesis most strongly in the brain. In case of ethanol we can observe approximately 10-times increase in the MT level and in case of morphine – approx. 15-times increase of MT level comparing to the level in the control group of animals.

In the liver the induction of the MT synthesis is rather moderate. In the ethanol intoxicated group the average MT level in that organ was 9.34 μg/g tissue (comparing to the MT level in the control group – it means almost 4-times increase). The same increase is observed in the morphine intoxicated group.

Metallothioneins are induced most weakly in kidneys. Both narcotic agents cause only approx. 2-times increase in the metallothionein level.

Although all tissues are able to synthesise metallothioneins, the main place of their synthesis is the liver. The experiments conducted on the animals show that heavy metals (e.g. cadmium) most strongly induce the MT synthesis in the liver (5, 6, 10, 15). Hamer (10) suggests that the metallothionein level is dependent on a tissue and the metal exposure time. Besides, the MT synthesis depends on the affinity of the tissue towards the appropriate metal (14).

The increase in the MT level in the brain in the ethanol and morphine intoxicated groups may be connected with the increase in the MT-III, located in the nevous system. The MT-III was induced under the influence of the narcotic agents used. Kelly et al. and Roesijadi (11, 14) suggest that metallothioneins may be one of the factors responsible for the pharmacologic agents resistance of the cell. Such increase in the MT level is observed in the cancer cells and this change correlates with the decreased adriamycine activity effect (12). The increase in the metallothioneins' level in the narcotic agents intoxicated groups may be the example of the similar mechanism.

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SUMMARY

Metallothioneins take part in homeostasis of the ions of metals which are necessary for proper metabolism of the organism (zinc, copper), biosynthesis regulation and zinc proteins activity as well as in the processes of detoxication of cells from toxic metals. Besides, they also protect cells from the reactive forms of oxygen, radiation, electrophilic pharmacological agents used in the cancer therapy, and the mutagenes.

The aim of the study was to determine the level of metallothioneins in tissue of rats intoxicated with ethanol and morphine. The experiment was to state whether the applied narcotic agents cause the change in the metallothionein's level in the liver, brain and kidneys of the intoxicated animals. The experiment was carried out on 6-month-old rats of Wistar strain, weighing 150-200g, which were given ethanol intragastrically and morphine intraperitoneally for 5 days. The metallothioneins level in tissues was determined by cadmium-hemoglobin affinity assay, using the cadmium isotope (^{109}Cd).

It was found that the level of metallothioneins in the group of animals intoxicated with ethanol was elevated (in kidneys it increased 1.5 times, in the liver – 3.5, and in the brain – 10 times). In the case of morphine-intoxicated rats the level of metallothioneins also increased in all the examined tissues; the increase was fifteenfold in the brain, threefold in the liver, and twofold in kidneys.

It results from the above studies that the narcotic agents used for the animals' intoxication produce the increase in the level of metallothioneins in all the examined rats' tissues. The examinations prove different force of metallothioneins induction according to a tissue.

Indukcja metalotionein za pomocą etanolu i morfiny

Metalotioneiny biorą udział w homeostazie jonów metali wymaganych w prawidłowym metabolizmie (cynk, miedź), regulacji biosyntezy oraz aktywności cynkoprotein, a także w procesach detoksykacji komórek z metali toksycznych. Oprócz tego białka te zabezpieczają komórki przed reaktywnymi formami tlenu, promieniowaniem jonizującym, środkami farmakologicznymi stosowanymi w terapii nowotworów oraz mutagenami.

Celem pracy było określenie poziomu metalotionein w tkankach szczurów intoksykowanych etanolem i morfiną. Eksperyment miał odpowiedzieć na pytanie, czy zastosowane środki narkotyczne powodują zmianę w poziomie metalotionein w wątrobie, mózgu oraz nerkach u intoksykowanych szczurów. Doświadczenie prowadzono na 6-miesięcznych szczurach (szczep Wistar), o wadze 150--200 g, którym przez 5 dni podawano dożołądkowo etanol oraz dootrzewnowo morfinę. Poziom metalotionein w tkankach oznaczono metodą izotopową, stosując ^{109}Cd.

W grupie zwierząt intoksykowanych etanolem stwierdzono podwyższony poziom metalotionein we wszystkich badanych tkankach (w nerkach – 1,5-krotnie, w wątrobie – 3,5-krotnie, a w mózgu – 10-krotnie). W grupie zwierząt intoksykowanych morfiną również stwierdzono podwyższony poziom metalotionein we wszystkich badanych tkankach: w mózgu – 15-krotnie, w wątrobie – 3-krotnie oraz w nerkach – 2-krotnie.

Z przeprowadzonych badań wynika, że zastosowane w intoksykacji zwierząt środki narkotyczne powodują wzrost poziomu metalotionein we wszystkich badanych tkankach u szczurów. Badania wskazują na różną siłę indukcji metalotionein w zależności od tkanki.