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Magnesium concentration in rat tissues receiving sex hormones and vitamin E

Magnesium plays an important role in regulation of various physiological processes in living organisms. It is a cofactor of many hundreds of enzymatic reactions. Magnesium also plays a part in protein and nucleic acids synthesis, DNA and RNA transcription and translation of messenger RNA. Moreover, it may have a critical role in the regulation of mitochondrial function (5, 13, 14, 16). In the organism, the influence on the maintenance of magnesium homeostasis have the following hormones: parathormone, calcitonin, insulin, glucagon, antidiuretic hormone, adrenaline and noradrenaline, aldosterone and sex hormones (13, 16). From among the vitamins the best known influence on magnesium economy has vitamin D_3 (4). Magnesium reabsorption in kidney stimulated by parathormone increases intestine absorption and increases magnesium releasing from bone. Similarly, but to a lower degree, calcitonin increases the magnesium reabsorption in the kidney. Insulin is a preventive hormone on magnesium status. It regulates magnesium delivery to cells and it facilitates magnesium transport by cell membranes. Glucagon exerts not large influence on the magnesium level, but it decreases magnesium kidney reabsorption, which was experimentally showed on rats. Adrenaline and noradrenaline stimulate magnesium loss with urine. The antidiuretic hormone and thyroid hormones affect similarly. Thyroid hormones decrease magnesium concentration mainly by increased demand for this element due to metabolism intensification. Aldosterone has only a little influence on the magnesium level. It increases magnesium loss by the kidney. It is a secondary effect of increased kidney filtration. Vitamin D_3 has a similar structure to steroid hormones. It influences magnesium intestinal absorption, but increases kidney magnesium loss. There is a correlation between magnesium or calcium levels and hormones, which increase calcium level and decrease magnesium level simultaneously (13, 14, 15).

Sex hormones, both in female and male, affect magnesium homeostasis, too. Hitherto prevailing studies showed the changes in this element concentration in female in other phase of menstrual cycle. It was noticed that estrogens and progesterone fulfil the role in regulation of the magnesium level (9, 10, 12). Similarly, testosterone influenced the magnesium level, too (7).

The aim of our study was to determine the influence of various testosterone and estradiol doses on magnesium concentration in rat tissues. Moreover, we analysed the effect of vitamin E applied with sex hormones on magnesium concentration in tested tissues of male and female rats.

MATERIAL AND METHODS

The experiment was conducted on 14-month-old Wistar rats, weighing 350–400 g. The animals were divided into 12 groups, 10 rats each. All animals were fed a standard diet (LSM dry food and redistilled drinking water). Food and water were given *ad libitum*. The groups 1–6 comprised females only and the groups 7–12 males only. The first and the seventh groups were the control groups. The other groups of animals received intragastric sex hormones suspended in a Tween 80-solution daily for 14 days. The second, third, fifth and sixth groups received estradiol, but the third and the sixth groups at the dose of 0.01 mg kg^{-1} b.w., whereas the third and the sixth group at the dose of 0.1 mg kg^{-1} b.w. The eighth, ninth, eleventh and twelfth group received testosterone, but the eighth and the eleventh group at the dose of 0.01 mg kg^{-1} b.w., while the ninth and the twelfth group at the dose of 0.1 mg kg^{-1} b.w. Additionally the groups 4–6 and 10–12 obtained vitamin E at the dose of 100 mg kg^{-1} of b.w.

After two weeks all rats were sacrificed under ketamine anaesthesia (1 ml of 5% ketamine via intraperitoneal injection). Following decapitation, the brain, liver, kidneys and heart were removed and homogenised in fourfold volumes of 100 mM Tris-HCl buffer pH 7.4. The homogenates were centrifuged 5000 x g by 20 min. The magnesium concentration was determined in obtained supernatants in the reaction with xylidine blue (Cormay-Mg). The results were submitted to statistical analysis with the Cochran-Cox test, accepting $p < 0.05$ as significant.

RESULTS

Estradiol administration to rats resulted in diminished of magnesium concentration in all the tested tissues (Table 1).

Table 1. Magnesium concentration (mg kg^{-1} of tissue) in female rat tissues receiving estradiol and estradiol with vitamin E

Tested tissue	Control X ± SD	Estradiol 0.01 mg kg^{-1} b.w. X ± SD	Estradiol 0.1 mg kg^{-1} b.w. X ± SD	Control with vitamin E X ± SD	Estradiol 0.01 mg kg^{-1} b.w. with vitamin E X ± SD	Estradiol 0.1 mg kg^{-1} b.w. with vitamin E X ± SD
Brain	4.31 ± 0.6	3.53 ± 0.4↓	1.99 ± 0.3↓*	3.45 ± 0.3	2.84 ± 0.3↓	2.52 ± 0.4↓*
Kidney	4.06 ± 0.6	2.69 ± 0.3↓*	2.18 ± 0.4↓*	4.02 ± 0.6	3.57 ± 0.5↓	2.38 ± 0.4↓*
Liver	5.95 ± 0.5	3.20 ± 0.3↓*	2.55 ± 0.4↓*	3.55 ± 0.4	3.11 ± 0.4↓	2.69 ± 0.5↓*
Heart	3.22 ± 0.4	2.78 ± 0.2↓	1.41 ± 0.5↓*	4.06 ± 0.7	3.09 ± 0.2↓*	1.85 ± 0.3↓*

Statistical significance vs. control* $p < 0.05$

The largest magnesium concentration decreases were observed in female groups receiving the higher estradiol dose (0.1 mg kg^{-1} b.w.). These changes were statistically significant in all the tested tissues as compared to controls. In the case of the administration of the lower estradiol dose (0.01 mg kg^{-1} b.w.) magnesium loss was observed in all the female tested tissues in comparison to control, but only in the kidney and liver it was statistically significant.

The vitamin E administration only to a little degree influenced magnesium concentration rise in control group with vitamin E in comparison to control. The estradiol administration, in both doses, with vitamin E, reduced magnesium concentration in the tested tissues. These results were statistically significant in all the tested tissues in the case of the higher estradiol dose with vitamin E. In the case of

the lower estradiol dose with vitamin E the magnesium level showed a tendency to decrease, but statistically significant difference was observed in the heart only.

The application of sex hormone (testosterone) to male rats altered magnesium concentration in the tested tissues (Table 2).

Table 2. Magnesium concentration (mg kg^{-1} of tissue) in male rat tissues receiving testosterone and vitamin E

Tested tissue	Control X ± SD	Testosterone 0.01 mg kg^{-1} b.w. X ± SD	Testosterone 0.1 mg kg^{-1} b.w. X ± SD	Control with vitamin E X ± SD	Testosterone 0.01 mg kg^{-1} b.w. with vitamin E X ± SD	Testosterone 0.1 mg kg^{-1} b.w. with vitamin E X ± SD
Brain	4.52 ± 0.6	3.50 ± 0.4↓	1.92 ± 0.3↓*	7.91 ± 0.8	4.55 ± 0.4↓*	2.87 ± 0.3↓*
Kidney	6.30 ± 0.7	4.80 ± 0.6↓*	3.40 ± 0.4↓*	7.67 ± 0.7	5.15 ± 0.5↓*	4.83 ± 0.6↓*
Liver	9.36 ± 0.9	4.69 ± 0.6↓*	3.07 ± 0.4↓*	8.30 ± 0.9	4.80 ± 0.5↓*	4.06 ± 0.5↓*
Heart	3.14 ± 0.3	2.48 ± 0.4↓	2.32 ± 0.3↓*	4.90 ± 0.4	4.34 ± 0.4↓	4.13 ± 0.4↓*

Statistical significance vs. control* $p < 0.05$

The decreases of magnesium concentrations were similar in the case of the higher dose of testosterone and the results were statistically significant in all the tested tissues. On administering the lower dose of testosterone there was observed a tendency to decrease in the magnesium level in the brain and heart, but in the kidney and liver magnesium loss was statistically significant in comparison to control.

The application of vitamin E together with testosterone altered the results to a small extent only. The administration of the higher dose of testosterone with vitamin E caused that magnesium concentration decreases were statistically significant in comparison to control with vitamin E. In the case of the lower dose of testosterone with vitamin E these results were similar in all the tested tissues except the heart. There was observed a tendency to magnesium concentration reduction.

DISCUSSION

Hormonal regulation of magnesium homeostasis plays the principal role in the management of this element (4, 16). It is similarly important because hypomagnesaemia may be the cause of many metabolic disturbances and diseases (1, 3, 6, 8, 17). The examined studies showed that female as well as male sex hormones influenced magnesium concentration in rat tissues. Estradiol, like testosterone, caused a decrease of magnesium concentration in the tested tissues. It is in accordance with results presented in literature by other authors. They have showed a decrease of magnesium concentration induced by sex hormones. The high estrogen or progesterone levels caused lower magnesium concentration in blood serum as well as in erythrocytes. Balance of hormone concentration, similarly as in the course of menstrual cycle in women may have influence on appearance of hypomagnesaemia and symptoms of it. In women, certain sufferings in the course of menstrual cycle such as migraines may be caused by lower concentration of magnesium. As a consequence, vasomotor disturbances occur (7).

The studies conducted on human blood serum showed that sex hormone estrogens as well as testosterone decreased ionised magnesium concentration and decreased total magnesium concentration. Sex hormones influenced the calcium to magnesium ratio, too (10, 11). Muneyyirci-Delale et al. have observed that progesterone may play an important role in regulation of the ionised magnesium level (12).

Our studies have showed that the decrease of tissues magnesium concentration and the influence of estradiol as well as testosterone were similar. That may be probably indicative of the increased magnesium loss by the organism or its displacement to other tissues. Vitamin E, as a small molecular antioxidant, plays the role in the human organism and it is known as fertility vitamin (2). Vitamin E applied with estradiol or testosterone did not show any greater influence on tissue magnesium concentration. Vitamin E, only to a little degree, influenced protectively magnesium concentration in the tested tissues. As an antioxidant, it exerts protective effect on the cells membrane, and this may cause limitation of magnesium outflow from cells and its loss. Further studies may explain mutual dependence in sex hormones action and vitamin E on the magnesium management.

CONCLUSION

1. Estradiol as well as testosterone diminished tissue magnesium concentration and the degree of reduction depended on the applied hormone dose.

2. Vitamin E applied with sex hormones decreases their negative influence on the magnesium level.

REFERENCES

1. Altura B. M., Altura B. T.: Magnesium in cardiovascular biology. Sci. Am., 2, 28, 1995.
2. Bielak E., Pasternak K.: Witaminy A, C, E – antyoksydantami. Probl. Piel., 1-2, 82, 2001.
3. Brown I. R. et al.: Sex difference in the relationship of calcium and magnesium excretion to glycaemic control in type 1 diabetes mellitus. Clin. Chim. Acta, 283, 119, 1999.
4. Durlach J.: Magnez w praktyce klinicznej. PZWL, Warszawa 1991.
5. Fawcett W. J. et al.: Magnesium – physiology and pharmacology. Br. J. Anaesth., 83, 2, 302, 1999.
6. Levin G. E. et al.: Tissue magnesium status in diabetes mellitus. Diabetologia, 21, 131, 1981.
7. Li W. et al.: Sex steroid hormones exert biphasic effects on cytosolic magnesium ions in cerebral vascular smooth muscle cells: Possible relationships to migraine frequency in premenstrual syndromes and stroke incidence. Brain Res. Bull., 54, 1, 83, 2001.
8. Ma J. et al.: Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin and carotid arterial wall thickness: The aric study. J. Clin. Epidemiol., 48, 7, 927, 1995.
9. Mc Nair P. et al.: Effect of menopause and estrogen substitutional therapy on magnesium metabolism. Miner. Electrolyte Metab., 10, 84, 1984.
10. Muneyyirci-Delale O. et al.: Sex steroid hormones modulate serum ionized magnesium and calcium levels throughout the menstrual cycle in women. Fertil. Steril., 69, 5, 958, 1998.
11. Muneyyirci-Delale O. et al.: Serum ionized magnesium and calcium in women after menopause: inverse relation of estrogen with ionized magnesium. Fertil. Steril., 71, 5, 869, 1999.
12. Muneyyirci-Delale O. et al.: Serum ionized magnesium and calcium and sex hormones in healthy young men: importance of serum progesterone level. Fertil. Steril., 72, 5, 817, 1999.
13. Pasternak K.: Magnez w fizjologii człowieka. Biul. Magnezol., 4, 2, 480, 1999.
14. Pasternak K.: Biopierwiastki w praktyce medycznej. Instytut Edukacji Zdrowotnej i Opieki Człowieka, AM Lublin, 2000.
15. Ryan M. F.: The role of magnesium in clinical biochemistry: an overview. Ann. Clin. Biochem., 28, 19, 1991.

16. Saris N. E. L et al.: Magnesium. An update on physiological, clinical and analytical aspects. Clin. Chim. Acta, 294, 1, 2000.
17. Skotnicki A. B.: Przeciwstresowe – kardioprotekcyjne właściwości magnezu. Probl. Lek., 31, 1, 3, 1992.

SUMMARY

Magnesium plays very important metabolic and regulatory roles in the animal organism. On this element tissues' concentration may have an influence other factors, such as sex hormones and vitamin E. The aim of our paper was to determine the influence of various testosterone doses given to male rats and various estradiol doses given to female rats on tissue magnesium concentration. Moreover, we analysed the influence of vitamin E applied with sex hormones on magnesium concentration in the tested groups of male and female rats. Testosterone was given to male rats at the doses of 0.01 mg kg^{-1} of b.w. and 0.1 mg kg^{-1} of b.w. Estradiol was given to female rats at the doses of 0.01 mg kg^{-1} of b.w. and 0.1 mg kg^{-1} of b.w. Some groups of animals additionally received vitamin E at the dose of 100 mg kg^{-1} of b.w. The experiment was conducted for two weeks and next the brain, liver, kidneys and heart were taken for analysis. Tissue magnesium concentration was studied. We showed that sex hormones (estradiol in females, testosterone in males) influenced magnesium concentration reduction in the tested tissues. This decrease depended on the dose of applied hormone and only insignificantly on its kind. It was showed that simultaneously applied vitamin E diminished the effect of sex hormones action on magnesium concentration in the tested tissues.

Stężenie magnezu w tkankach szczurów otrzymujących hormony płciowe i witaminę E

Magnez spełnia w organizmie zwierzęcym wiele ważnych funkcji metabolicznych i regulacyjnych. Na jego stężenie w tkankach mogą wpływać różne czynniki, w tym również hormony płciowe i witamina E. Celem naszej pracy było określenie wpływu testosteronu podawanego samcom oraz estradiolu podawanego samicom na tkankowe stężenia magnezu. Dodatkowo analizowano wpływ witaminy E podawanej wraz ze stosowanymi hormonami na stężenie magnezu w badanych grupach samców i samic. Testosteron podawano samcom w dawkach 0.01 mg kg^{-1} m.c. i 0.1 mg kg^{-1} m.c., natomiast estradiolu samicom w dawkach 0.01 mg kg^{-1} i 0.1 mg kg^{-1} m.c. W grupach samców i samic podawano również witaminę E w dawce 100 mg kg^{-1} m.c. Doświadczenie przeprowadzano przez okres 2 tygodni, a następnie pobierano mózg, wątrobę, nerki i serce w celu oznaczenia tkankowego stężenia magnezu. Wykazano, że podawanie hormonów płciowych (testosteronu u samców, estradiolu u samic) wpływa na obniżenie stężenia magnezu w badanych tkankach. Obniżenie stężenia magnezu zależy od dawki podawanego hormonu, a tylko nieznacznie od jego rodzaju. Jednoczesne stosowanie witaminy E zmniejsza efekt działania hormonów płciowych na stężenie magnezu w badanych tkankach.