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[1]Palladin Institute of Biochemistry, National Academy of Science of Ukraine, Kyiv, Department of Molecular Biology,
[2]*SI* Institute *for* Occupational Medicine Academy of Medical Sciences of Ukraine, Kyiv Ukraine;
ominchenko@yahoo.com

IRYNA BOZHKO[1], DMYTRO MINCHENKO[1],
VALENTYNA MYKHALCHENKO[1], SVITLANA IVASHKEVYCH[1],
OLENA APYKHTINA, NATALIYA DMYTRUKHA,
ISAAC TRAKHTENBERG[2], OLEKSANDR MINCHENKO

Effect of manganese, cadmium, plumbum and mercury ions on the expression of SNF1/AMP-activated protein kinase in the rat liver, lungs, kidney and heart

Wpływ jonów manganu, kadmu, ołowiu i rtęci na ekspresję kinazy białkowej aktywowanej SNF1/AMP w wątrobie, płucach, nerce i sercu szczurów

INTRODUCTION

Most physiological and metabolic processes in organism are controlled by different regulatory factor network, which includes a lot of protein kinases, protein phosphatases and transcription factors [3,8,13]. These factors are key regulators of metabolism both in normal and different pathological conditions, its circadian character of regulation [9,11]. Moreover, disturbances in the expression of these regulatory factors were observed in different pathological conditions and they may be responsible for the appearance and progression of malignant tumors [2,10,14,15]. SNF1/AMP-activated protein kinase (SNARK) is a member of AMPK kinases which are related to serine/threonine protein kinases [4,5]. SNARK activity is regulated by glucose- or glutamine-deprivation, induction of endoplasmic reticulum stress by homocysteine or DTT, elevation of cellular AMP and/or depletion of ATP, hyperosmotic stress, salt stress, ultraviolet B radiation and oxidative stress caused by hydrogen peroxide. Moreover, the regulation of SNARK activity in response to cellular stresses depends greatly upon cell type. These observations support the role for SNARK as a molecular component of the cellular stress response [4,5].

Snark(+/-) mice exhibited mature-onset obesity and related metabolic disorders [12]. Obesity is regarded as a risk factor for colorectal cancer. To investigate whether Snark deficiency is involved in tumorigenesis in the large intestine, obese Snark(+/-) mice were treated with a chemical carcinogen, azoxymethane. The incidences of both adenomas and aberrant crypt foci were significantly higher in

Snark(+/-) mice than in their wild-type counterparts, suggesting that Snark deficiency contributed to the early phase of tumorigenesis via obesity-dependent and -independent mechanisms [12].

Expression SNARK kinase significantly changed in different tissues of rats treated with ecotoxicant methyl tertbutyl ether [6]. Cadmium, plumbum and mercury are widely distributed heavy metals which have toxic health effects but there are no data about their possible effect on SNARK kinase.

In this work we studied the expression of protein kinase SNARK in the liver, lung, heart and kidney from rats treated during 1 month with manganese, cadmium, plumbum and mercury ions.

MATERIAL AND METHODS

The experiments were performed on male Wistar rats (initial body weight 160–180 g). Five groups of animals were treated by daily intraperitoneal injection of manganese sulfate (8,5 mg/kg), cadmium sulfate (0.8 mg/kg), plumbum acetate (1.53 mg/kg), mercury chloride (0,19 mg/kg) or saline (control group) during one month (28 injections). Expression of protein kinase SNARK mRNA in the liver, lungs, kidney and heart was measured using quantitative (real time) polymerase chain reaction. RNA was extracted using Trizol reagent (Invitrogen, USA) [7]. Expression of SNARK mRNA in different rat organs was measured by polymerase chain reaction using HotStarTaq Master Mix Kit ("QIAGEN", Germany) and "MasterCycler Personal" ("Eppendorf", Germany) and quantitative polymerase chain reaction using SYBRGreen Mix (AB gene, Great Britain) and „Stratagene Mx 3000P cycler" (USA). QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis. Quantitative PCR was performed in triplicate. The analysis of quantitative PCR was performed using a special computer program "Differential expression calculator" and statistic analysis – in Excel program.

RESULTS

We showed that the expression of protein kinase SNARK mRNA is significantly decreased in the lung, heart and kidney in rats treated with manganese (Fig. 1 and 2). Significant induction of protein kinase SNARK mRNA expression was observed in the liver and lungs in rats treated with plumbum (+112 and 77%, correspondingly) and mercury (+105 and 102%, correspondingly), but cadmium enhances this mRNA expression (+75%) in the liver only (Fig. 3). At the same time, the expression of kinase SNARK mRNA in the heart is decreased in animals treated with manganese, plumbum and mercury salts (-32, -25 and -44%, correspondingly; Fig. 4). Moreover, the levels of protein kinase SNARK mRNA expression were unchanged in rats treated with cadmium, plumbum and mercury salts (Fig. 4).

Thus, cadmium, magnesium, plumbum and mercury ions can affect protein kinase SNARK expression in different rat vital organs and they change important regulatory mechanisms mediated by this protein kinase.

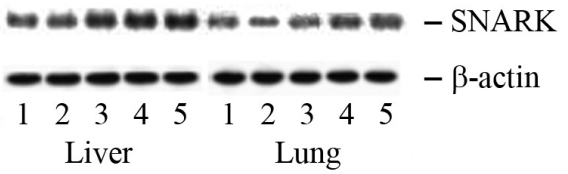

Fig.1. SNARK mRNA expression in rat liver and lung measured by polymerase chain reaction (PCR): effect of manganese (2), cadmium (3), plumbum (4) and mercury (5). In Fig. 1 and 2: β-actin mRNA expression was used as control of RNA quantity; 1 – control

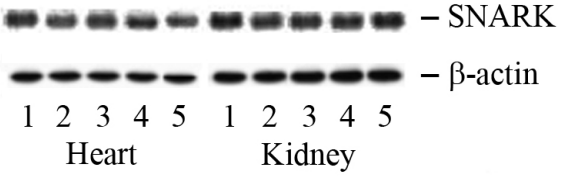

Fig. 2. Expression of SNARK mRNA in rat heart and kidney measured by PCR: effect of manganese (2), cadmium (3), plumbum (4) and mercury (5)

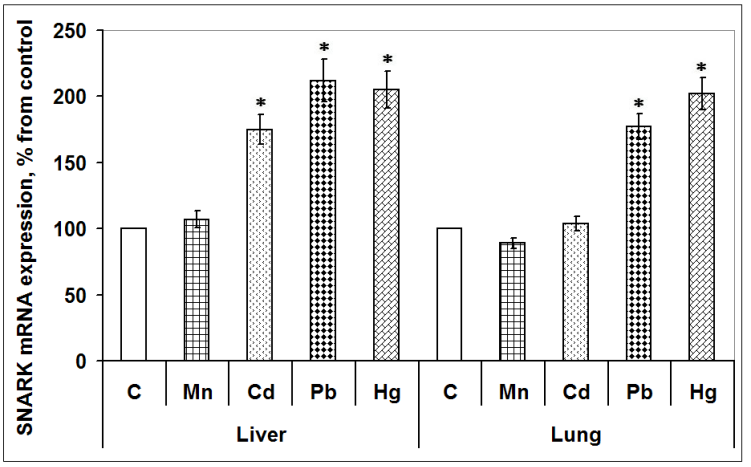

Fig. 3. SNARK mRNA expression in rat liver and lung measured by quantitative PCR. In Fig. 3 and 4: Values of SNARK mRNA expressions were normalized to β-actin mRNA expression; * $P < 0.05$ as compared to control; $n = 3$

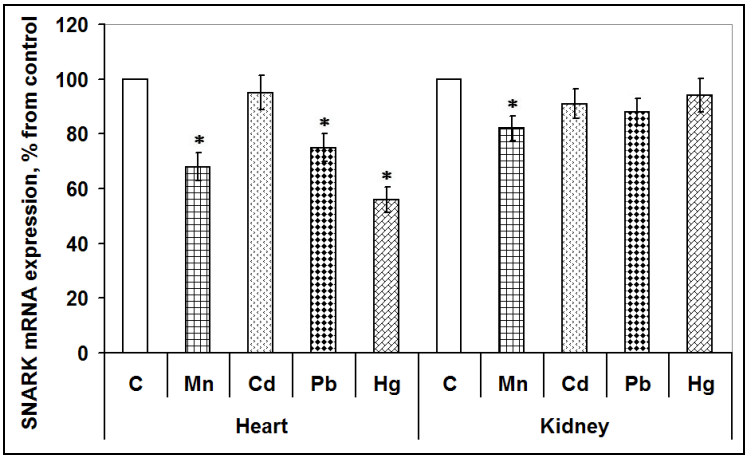

Fig. 4. SNARK mRNA expression in rat heart and kidney measured by quantitative PCR

DISCUSSION

Results of this investigation show that the effect of different heavy metal ions has different action on the expression of SNF1/AMP-activated protein kinase in an organ specific manner. These results correlate to data provided by Lefebvre and Rosen [5]. They showed that the regulation of SNARK activity in response to cellular stresses depends greatly upon cell type. Because protein kinase SNARK is a molecular component of the cellular stress response [1,5], it is possible that the effect of heavy metal ions on SNARK expression is a result of cellular stresses induced by these compounds and depends greatly upon heavy metal ion type. Results of this investigation clarify some molecular mechanisms of several heavy metals action on the regulation of metabolism and its bioinsequrity. However, further investigations of the mechanism by which heavy metal ions affects SNARK expression and biologic significance of heavy metal induced alteration in SNARK expression are needed. The stage is now set for the elucidation of the molecular mechanisms responsible for these important SNARK responses to heavy metal ions action.

CONCLUSIONS

Results of our investigation clearly demonstrated that cadmium, magnesium, plumbum and mercury ions can affect some important regulatory mechanisms which control cell metabolism via protein kinase gene expression, in particular, protein kinase SNARK in different rat vital organs. Disturbance of this gene expression can destroy the cellular signal pathways and leads to the development of pathological processes.

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SUMMARY

The expression of SNF1/AMP-activated protein kinase (SNARK) mRNA was significantly decreased in the lung, heart and kidney in rats treated with manganese. Significant induction of protein kinase SNARK mRNA expression was observed in the liver and lungs in rats treated with plumbum and mercury, but cadmium enhances this mRNA expression in the liver only. At the same time the expression of kinase SNARK mRNA in the heart is decreased in animals treated

with manganese, plumbum and mercury salts. Thus, results of this investigation demonstrated that cadmium, manganese, plumbum and mercury can affect some important regulatory mechanisms which control cell metabolism at the SNARK protein kinase level.

Key words: SNF1/AMP-activated protein kinase (SNARK), manganese, cadmium, plumbum, mercury, rats

STRESZCZENIE

Ekspresja mRNA kinazy białkowej aktywowanej SNF1/AMP była istotnie obniżona w płucach, sercu i nerce szczurów otrzymujących mangan. Obserwowano znaczną indukcję ekspresji mRNA kinazy białkowej SNARK w wątrobie i płucach szczurów otrzymujących ołów i rtęć, ale kadm zwiększał ekspresję tego mRNA jedynie w wątrobie. W tym samym czasie ekspresja mRNA kinazy SNARK w sercu była zmniejszona u zwierząt otrzymujących sole manganu, ołowiu i rtęci. Wyniki tego badania wskazują zatem, że kadm, mangan, ołów i rtęć mogą wpływać na niektóre ważne mechanizmy regulacyjne, które kontrolują metabolizm komórkowy na poziomie kinazy białkowej SNARK.

Słowa kluczowe: kinaza białkowa aktywowana SNF1/AMP (SNARK), mangan, kadm, ołów, rtęć, szczury