

PREVENTION OF MERCURY-INDUCED EXCITOTOXICITY IN PRESYNAPTIC BRAIN NERVE TERMINALS WITH CARBON DOTS

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Mercury is hazardous neurotoxicant. Carbon-containing nanoparticles (CNPs) are promising in nanotechnology. It was shown that HgCl_2 starting from 5 μM caused a concentration-dependent increase in the extracellular L-[^{14}C]glutamate level in nerve terminals resulted from weak functioning of glutamate transporter, and so significantly decreased L-[^{14}C] glutamate uptake. Combined effects of Hg^{2+} and CNPs obtained by heating of citric acid and urea were analysed. CNPs were able to mitigate in an acute manner excitotoxic Hg^{2+} -induced increase in the extracellular L-[^{14}C]glutamate level in nerve terminals by 37%, thereby being a provisional Hg^{2+} scavenger. Besides biotechnological implementation of data, developed approach can be applicable for monitoring capability of different particles and compounds to mitigate Hg^{2+} -mediated threat.

Xenobiotic metal mercury is one of the major crucial pollutants of global public health concerns according to the World Health Organization assessment [1, 2]. Mercury exists in elemental, inorganic, and organic forms [1, 2]. This metal is available in the environment coming from natural and anthropogenic sources. Mercury contaminates the soil, air and surface waters and may enter human organism [3–5]. The central nervous system is targeted by mercury [1].

Carbon-containing nanoparticles are promising in nanotechnology and due to their surface properties can be used for adsorption of heavy metals.

The *aim* of this study was to analyse a capability of carbon-containing nanoparticles (CNPs) obtained by heating of organics, to influence mercury-induced neurotoxicity in biological system, such as presynaptic rat cortex nerve terminals.

Methods. CNPs were obtained using method described in [6] by the combustion of citric acid and urea.

The cortex nerve terminals isolated from Wistar rats were used in the experiments. [^{14}C] glutamate uptake and release in the nerve terminals were monitored using a radiolabelled assay. In particular, rat brain nerve terminals (synaptosomes) were isolated from the rat cortex. The cortex regions were rapidly removed and homogenized in the ice-cold solution consisted of: sucrose 0.32 M; HEPES-NaOH 5 mM, pH 7.4; EDTA 0.2 mM. One synaptosomal preparation was isolated from one rat. The synaptosomes from brain homogenate were obtained according to the procedure proposed by Cotman with minor modifications [7] by differential centrifugation and Ficoll-400 density gradient centrifugation. The concentrations of proteins were monitored according to Larson.

To measure the uptake of L-[^{14}C] glutamate, the synaptosomal suspension was pre-incubated in the standard saline solution. Then, HgCl_2 was applied to the synaptosomal incubation media, and synaptosomes were further incubated for 6 min before starting the uptake, which in turn was initiated by the application of the aliquots of non-radiolabelled L-glutamate (10 μM) supplemented with L-[^{14}C] glutamate, 420 nM, 0.1 $\mu\text{Ci/ml}$, and then the synaptosomes were incubated at 37 °C during 1 min to measure the initial rate of L-[^{14}C] glutamate uptake. L-[^{14}C] glutamate uptake was monitored with liquid scintillation counting using the ACS scintillation cocktail, 1.5 ml [8].

To measure the extracellular level of L-[^{14}C] glutamate, the synaptosomes were pre-incubated at 37 °C during 10 min to restore the ion gradients, and after that they were loaded with

L-[^{14}C] glutamate, 1 nmol per mg of protein, 238 mCi/mmol, in the standard saline solution at 37 °C during 10 min according to [9]. Total synaptosomal content of L-[^{14}C] glutamate was equal to 200000 ± 15000 cpm/mg protein.

Results. In the first sets of the experiments, Hg^{2+} effects on the extracellular level of L-[^{14}C] glutamate were assessed in nerve terminal preparations (Fig.1). It was shown a mercury-induced excitotoxic increase in the ambient level of L-[^{14}C] glutamate in nerve terminal preparations.

In the second sets of the experiments (Fig. 2), it was demonstrated that Hg^{2+} decreased the initial rate and accumulation of L-[^{14}C] glutamate by nerve terminals starting from a concentration of 10 μM .

Therefore, it was shown that a mercury-induced excitotoxic increase in the ambient level of L-[^{14}C] glutamate in nerve terminal preparations (Fig. 1) resulted from weak functioning of glutamate transporter, and so significantly decreased L-[^{14}C] glutamate uptake (Fig. 2).

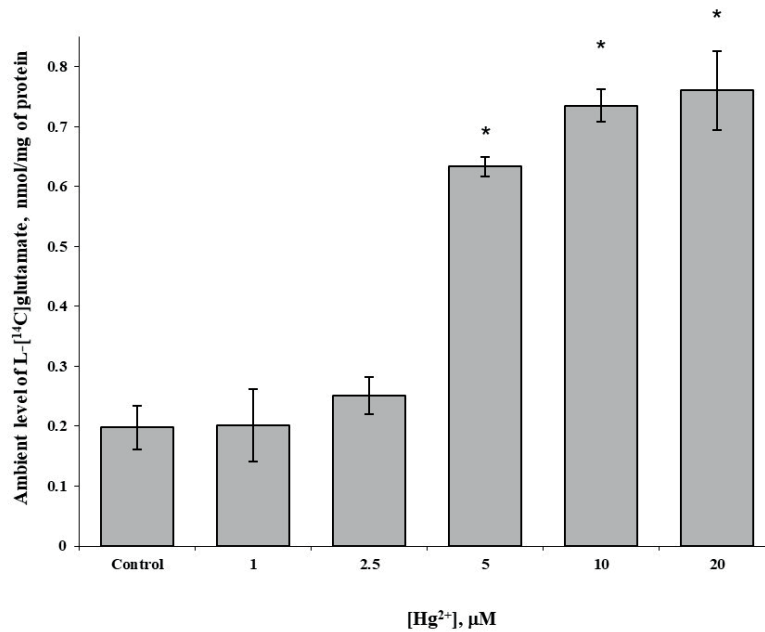


Fig. 1. The extracellular level of L-[^{14}C] glutamate in nerve terminal preparations in the presence of HgCl_2 within the concentration range from 0.5 to 20 μM
* — $P \leq 0.05$ as compared to the control; $n = 6$

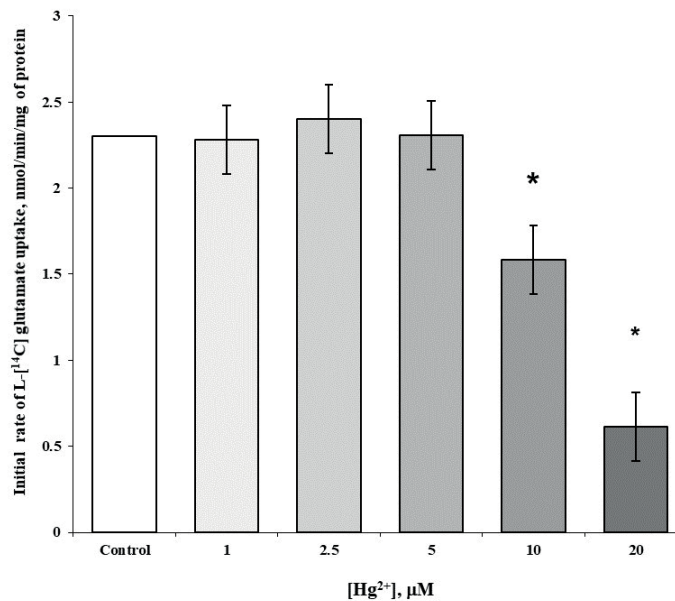


Fig. 2. The initial rate of L-[^{14}C] glutamate uptake by nerve terminals in the presence of HgCl_2 at different concentrations
* — $P \leq 0.05$ as compared to the control; $n = 10$

In the third sets of the experiments, it was shown that CNPs from heating of citric acid/urea mitigated an excitotoxic mercury-induced increase in the extracellular level of L-[¹⁴C] glutamate in nerve terminal preparations. The latter was equal to 0.425 ± 0.023 nmol/mg of proteins after combined application of HgCl₂ (5 μM) and CNPs (1 mg/ml) ($P \leq 0.05$ as compared to effect of Hg²⁺ per se; $n = 6$) and 0.460 ± 0.017 nmol/mg of proteins after combined application of HgCl₂ (5 μM) and CNPs (10 mg/ml) ($P \leq 0.05$ as compared to effect of Hg²⁺ per se; $n = 6$).

Therefore, CNPs were able to mitigate in an acute manner excitotoxic Hg²⁺-induced increase in the extracellular L-[¹⁴C]glutamate level in nerve terminals by 37%, thereby being a provisional Hg²⁺ scavenger.

Conclusions. CNPs can mitigate Hg²⁺-induced excitotoxicity in nerve terminals. Taking into account this fact, it can be assumed that these nanoparticles can be used as Hg²⁺ adsorbent in the human organism. Besides biotechnological implementation of data, developed approach can be applicable for monitoring capability of different particles and compounds to mitigate Hg²⁺-mediated threat.

Key words: mercury; glutamate; neurotoxicity; nerve terminals; synaptosomes; carbon nanoparticles.

Authors' Contribution

M. Driuk — synthesis of CNPs; N. Krisanova, N. Pozdnyakova, M. Dudarenko, A. Pastukhov; M. Driuk — isolation of synaptosomes; L-[¹⁴C]glutamate experiments; figure preparation; and paper draft preparation; T. Borisova—funding acquisition; development of research direction; project management and paper writing.

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