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INTERMINGLED MODULATORY AND NEUROTOXIC EFFECTS OF THIMEROSAL AND MERCURIC IONS ON ELECTROPHYSIOLOGICAL RESPONSES TO GABA AND NMDA IN HIPPOCAMPAL NEURONS

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The organomercurial, thimerosal, is at the center of medical controversy as a suspected factor contributing to neurodevelopmental disorders in children. Many neurotoxic effects of thimerosal have been described, but its interaction with principal excitatory and inhibitory neurotransmitter systems is not known. We examined, using electrophysiological recordings, thimerosal effects on GABA and NMDA-evoked currents in cultured hippocampal neurons. After brief (3 to 10 min) exposure to thimerosal at concentrations up to 100 μ M, there was no significant effect on GABA or NMDA-evoked currents. However, following exposure for 60-90 min to 1 or 10 μ M thimerosal, there was a significant decrease in NMDA-induced currents ($p < 0.05$) and GABAergic currents ($p < 0.05$). Thimerosal was also neurotoxic, damaging a significant proportion of neurons after 60-90 min exposure; recordings were always conducted in the healthiest looking neurons. Mercuric chloride, at concentrations 1 μ M and above, was even more toxic, killing a large proportion of cells after just a few minutes of exposure. Recordings from a few sturdy cells revealed that micromolar mercuric chloride markedly potentiated the GABAergic currents ($p < 0.05$), but reduced NMDA-evoked currents ($p < 0.05$). The results reveal complex interactions of thimerosal and mercuric ions with the GABA_A and NMDA receptors. Mercuric chloride act rapidly, decreasing electrophysiological responses to NMDA but enhancing responses to GABA, while thimerosal works slowly, reducing both NMDA and GABA responses. The neurotoxic effects of both mercurials are interwoven with their modulatory actions on GABA_A and NMDA receptors, which most likely involve binding to these macromolecules.

Key words: GABA_A receptors, neurotoxicity, NMDA receptors, patch-clamp, thimerosal, mercuric ions, hippocampal neurons

INTRODUCTION

Thimerosal (THIM), an organomercurial containing approximately 49% of mercury by weight, has been added for decades to medicinal products, including pediatric vaccines, without being sufficiently tested for its safety. This is surprising in view of the fact that all mercurials are highly toxic, particularly to developing organisms. In the past decade concerns emerged about the possibility that THIM from vaccines might contribute to certain neurodevelopmental disorders in children, which prompted its recent removal from most pediatric vaccines in the Western countries (7, 19). Unfortunately, it is still added to pediatric vaccines in less developed countries, including Poland, potentially damaging the health of children.

THIM is metabolized in the body to ethyl mercury (EtHg) and subsequently to inorganic mercury forms, which accumulate in tissues of vital organs, including the brain (22). Information about neurochemical and neurotoxic effects of THIM is still limited, but the existing data indicate that in pharmacodynamics and toxicity THIM/EtHg does not differ significantly from methyl mercury (MeHg), which has been studied more

extensively, although these compounds differ somewhat in pharmacokinetics (8).

Several studies documented that the neurotoxic effects of mercurials involve glutamate-mediated excitotoxicity, due to their ability to inhibit uptake of glutamate in astrocytes, resulting in an increase of the extracellular level of this excitatory amino acid (1, 4, 14). Excessive synaptic activity of glutamate may lead to excitotoxicity. Mercurials may interact as well with the glutamate receptors. MeHg has been shown to alter gene expression for the NMDA receptors (16) and to inhibit NMDA receptor binding *in vitro* (23), but in electrophysiological recordings both MeHg and HgCl₂ were without apparent rapid modulatory effect on the NMDA-induced currents in neurons (25). Equally ambiguous are the effects of mercurials on function of GABA_A receptors. Electrophysiological studies demonstrated that both MeHg and inorganic Hg interact with neuronal GABA_A receptors, albeit in opposite directions, as HgCl₂ potentiated the GABAergic currents, whereas MeHg decreased them (11, 20). An *in vivo* study showed an increased number of benzodiazepine receptors in rat brain, three days after acute MeHg administration (9).