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Stimulus-evoked glutamate release is diminished by acute exposure to uranium *in vitro*

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Abstract

Uranium is used in civilian applications, in the manufacture of nuclear fuel, and by the military for munitions and armament, but little information is available on its neurotoxicity. Neurological dysfunctions have been observed after chronic exposure in both animals and humans, but the actions of acute exposure on amino acid neurotransmission have not been investigated. The following study was performed to examine the effects of uranyl ion (UO_2^{+2}) on hippocampal glutamatergic and GABAergic function as possible bases for the neurotoxicity and to assess the direct effects on the exocytotic process. Nominal UO_2^{+2} concentrations were applied to superfused hippocampal synaptosomes to permit estimation of the metal's potency on endogenous transmitter release in the presence and absence of Ca^{+2}. K^+-evoked glutamate release was diminished in the range of 10 nM–316 μM UO_2^{+2}, resulting in an IC_{50} of 1.92 μM. In contrast, the potency of UO_2^{+2} to decrease stimulated GABA release was reduced, producing an $IC_{50} \approx 2.6$ mM. In the absence of Ca^{+2} in the superfusion medium there was no systematic change in the magnitude of glutamate or GABA release, suggesting that UO_2^{+2} does not possess Ca^{+2}-mimetic properties. The inhibitory potency of UO_2^{+2} on glutamate release is similar to the potencies of other multivalent metal ions, suggesting by inference an action exerted on voltage-sensitive Ca^{+2} channels. The bases for the reduced potency to inhibit GABA release is not known, but differential sensitivity to other heavy metals has been reported for glutamate and GABA neurotransmission. These findings indicate a profile of neurotoxicity not unlike that of other metal ions, and indicate the importance of extending subsequent studies to chronic exposure models.

Keywords: Uranyl ion; Hippocampus; Acute exposure; Glutamate release; GABA release; Calcium

1. Introduction

Uranium is a natural component of the earth's crust and a member of the actinide group of elements. The increasing use of uranium in industrial and military processes has resulted in increasing exposure to the metal, including via uptake into plants used as food sources [5]. The metal is used in some civilian applications, serving as weights and counterweights in ships and aircraft, shields for irradiation units in hospitals, and as transport containers for radioisotopes. Depleted uranium (DU) is a radioactive by-product of the enrichment process used to make nuclear fuel rods and nuclear bombs, but also is utilized by the military in munitions and armament because of its high density [1]. This form of uranium contains less of the highly radioactive isotope ^{235}U (0.3–0.5%) than does natural uranium (0.7%), but all forms of the element are chemically and physically identical.

The toxicity of uranium is largely associated with its chemical properties. Regardless of the form of the metal or its route of absorption, uranium forms a uranyl ion (UO_2^{+2}) in the systemic circulation that complexes with bicarbonate, citrate, or plasma proteins such as albumin [6,8,20]. It is deposited in various tissues such as kidney and liver, but approximately 85% of it ends up in bone [7], a distribution similar to that of other heavy metals such as lead [11]. Inhalation of uranium dust as oxides can solubilize in pulmonary alveoli and serve as an extended exposure source, while oral ingestion can occur from uranium-containing soil, dust or water [2].

In general, little information is available on the neurotoxic properties of uranium. A small number of studies have described the neurological signs resulting from acute exposure [e.g., [17]], but the exposure levels utilized were typically orders of magnitude greater than levels of current environmental or military relevance.

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Of greater interest are recent reports concerning chronic DU exposure in animals. The metal crosses the blood-brain barrier [9,12,15], and accumulates after chronic exposure via UO_2 inhalation [14], intramuscular DU pellet implantation [15], or UO_2^{+2} administration in drinking water [3,4]. Male rats receiving 150 mg UO_2^{+2}/L in drinking water for 2 weeks or 6 months exhibited gender-specific increases in open-field behaviors [3], and exposure to 40 mg UO_2^{+2}/L drinking water up to 9 months resulted in decreases in indices of regional brain dopaminergic and serotoninergic neuronal activity [4]. Pellmar et al. [16] investigated hippocampal neurophysiology employing chronic DU pellet implantation, and found that synaptic potentials in slices from exposed rats were less capable of eliciting population spikes than in slices from control animals, suggesting decreased neuronal excitability. Kidney toxicity was not evident in these animals. These observations were reinforced by the work of McDiarmid et al. [13] on a small group of Gulf War veterans retaining fragments of DU shrapnel 7 years after injury. Neurocognitive evaluations demonstrated a statistical relationship between urinary uranium levels and lowered performance on tests assessing performance efficiency. Again adverse effects in the kidney were not present. These reports clearly indicate that uranium crosses the blood-brain barrier in sufficient quantities to produce neurotoxicity, possibly without signs of renal dysfunction.

The following study was conducted to examine the effects of UO_2^{+2} on hippocampal glutamatergic and GABAergic function as possible bases for the behavioral and physiological changes described above. Acute exposure was utilized to identify the direct effects of UO_2^{+2} on the exocytotic process and provide a foundation for evaluating the effects of chronic uranium exposure in subsequent studies. A range of UO_2^{+2} concentrations were applied to hippocampal synaptosomes via the superfusing buffer to permit estimation of the inhibitory/stimulatory potency on the endogenous transmitter release component under study.

2. Methods

2.1. Animals

Male Sprague-Dawley rats were obtained at 70–80 days of age (Harlan, Indianapolis, IN) and housed in a vivarium with free access to tap water and standard laboratory chow. Lights in the animal room were set on a 12:12 cycle with temperature maintained at 24±1 °C. Animals were allowed to acclimate to the housing room for at least one week prior to inclusion in the study. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria and are consistent with the NIH *Guide for the Care and Use of Laboratory Animals*.

2.2. Synaptosomal preparation

After euthanasia, hippocampi were harvested in a cryostat at −2 °C and disrupted in ice-cold 10 mM HEPES-0.32 M sucrose buffer (pH 7.4) in a glass Potter–Elvehjem homogenizer with teflon pestle. Homogenates were pooled and centrifuged for 2 min at 3000 ×*g* at 4 °C, and the supernatant transferred to a clean tube and centrifuged again for 12 min at 14,600 ×*g* at 4 °C, resulting in isolation of a synaptosomal pellet. The pellet was resuspended in HEPES-sucrose buffer and stored on ice while total protein concentration was determined via the bicinchoninic acid assay [19]. Synaptosomes were then diluted to 1.0 mg protein/ml with an isotonic HEPES buffer (containing in mM: NaCl 132, KCl 1, $MgCl_2$ 1, $CaCl_2$ 1.3, glucose 10, HEPES 10, and 0.1% bovine serum albumin (BSA); bubbled in 99.9% O_2, pH 7.40) and incubated for 30 min at 37 °C. Carbonate and phosphate salts were not used in the buffer because of the ready formation of uranyl precipitates at neutral or alkaline pH.

2.3. Superfusion of synaptosomes

The synaptosomes were centrifuged for 30 s at 15,800 ×*g*, and after removal of the supernatant resuspended to 4.0 mg/ml in the above HEPES buffer without BSA, and containing a glutamate reuptake blocker − 0.5 mM DL-threo-β-hydroxyaspartic acid (Sigma Chemical, St. Louis, MO). To assess Ca^{+2}-independent release Ca^{+2} was replaced in the buffer by Mg^{+2} (final concentration 2.3 mM) and a Ca^{+2} channel antagonist (0.2 mM methoxyverapamil) added. While DU exposure is an important component of the rationale for this investigation, these experiments utilized native uranium salts. Thus, exposure solutions also contained uranium oxynitrate (Noah Technologies, San Antonio, TX) at concentrations ranging from 0–316 μM. These concentrations spanned the range of minimally effective inhibition of transmitter release to the limits of UO_2^{+2} solubility. 800 μg of synaptosomal protein was added to each superfusion chamber (Brandel, Gaithersburg, MD), and flow initiated with the same HEPES buffer at 0.6 ml/min. Flow was maintained for 30 min and then baseline samples were collected at 2 min intervals. During perfusate collection superfusion was switched for 2 min to the same HEPES buffer containing 31 mM KCl (Na^+ reduced to maintain isotonicity), and then returned to the normal buffer to re-establish the baseline. Thus, synaptosomes were exposed to UO_2^{+2} for 35–40 min before the depolarizing stimulus was applied.

The chemical species of uranium present in the superfusion buffer are not known. The UO_2^{+2} ion – the most common form produced in the body from all forms of the metal – is amphoteric and reacts with acids and bases, resulting in positively and negatively charged species. Theoretical models of uranium speciation in biological fluids have been developed [e.g., [21]], but do not include cerebrospinal fluid or physiological buffers such as the one employed in the current study. In a simple aqueous system at mildly alkaline pH, UO_2OH^+ is the prominent component [21], but in more complex ionic media diuranate ion ($U_2O_7^{-2}$) can be the primary form under alkaline conditions [22].

2.4. Chromatography

After superfusion was complete, each sample was prepared for HPLC analysis by derivatization using 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (Waters Corp., Milford, MA). The derivatives were quantified using binary gradient liquid chromatography with fluorescence detection (excitation – 250 nm and emission – 395 nm). Eluent A consisted of sodium