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NeuroToxicology xxx (2005) xxx–xxx

NeuroToxicology

Enriched But Not Depleted Uranium Affects Central Nervous System In Long-Term Exposed Rat

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Received 8 February 2005; accepted 22 April 2005

Abstract

Uranium is well known to induce chemical toxicity in kidneys, but several other target organs, such as central nervous system, could be also affected. Thus in the present study, the effects on sleep–wake cycle and behavior were studied after chronic oral exposure to enriched or depleted uranium. Rats exposed to 4% enriched uranium for 1.5 months through drinking water, accumulated twice as much uranium in some key areas such as the hippocampus, hypothalamus and adrenals than did control rats. This accumulation was correlated with an increase of about 38% of the amount of paradoxical sleep, a reduction of their spatial working memory capacities and an increase in their anxiety. Exposure to depleted uranium for 1.5 months did not induce these effects, suggesting that the radiological activity induces the primary events of these effects of uranium.

Keywords: Enriched uranium; Depleted uranium; Sleep; Memory; Anxiety; Chronic exposure

INTRODUCTION

Uranium is an alpha-emitter radioactive element from the actinide group. It is not only a radiotoxicant but also a heavy metal with chimiotoxicant properties (Priest, 2001). The kidney and bone are the primary reservoirs for uranium and the kidney is the most sensitive target organ for uranium toxicity (Diamond et al., 1989; Gilman et al., 1998). Although the nephrotoxicity of uranium has been well documented (Gilman et al., 1998; Taulan et al., 2004), few studies have reported toxic effects of uranium exposure on the central nervous system (CNS). In humans, states of depression or agitation were described after contamination by industrial uranium compounds as early as 1949 (Howland, 1949). More recently, neurocognitive impairments have been reported in soldiers injured with depleted uranium fragments received during the Gulf war (McDiarmid et al., 2000). In animals, previous studies have demonstrated that uranium can cross the blood-brain barrier (Lemercier et al., 2003) and may accumulate in the brains of rats receiving depleted uranium pellets implanted sub-cutaneously (Pellmar et al., 1999a). In another study, Pellmar et al. reported also electrophysiological disturbances in hippocampus slices isolated from rats embedded with depleted

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doi:10.1016/j.neuro.2005.05.005

uranium fragments for 6 and 12 months (Pellmar et al., 1999b). All these data suggested a possible neurological toxicity of chronic uranium exposure.

The key question is to determine if a chronic accumulation of low concentration of uranium in brain could induce neurophysiological and behavioral changes. Three neurobiological experiments were therefore performed, measuring respectively the electrical activity of the brain and the sleep–wake stages, the spatial working memory capacities and the anxiety-like behaviour after a chronic ingestion of enriched or depleted uranium exposure via drinking water during 1.5 months.

MATERIAL AND METHODS

The study was conducted in accordance with French legislation concerning the protection of animals used for experimental purposes. All procedures were performed by scientists certified by the French Ministry of Agriculture (license of first author no. 007310 delivered on 29th November 1996).

Contamination

Sprague–Dawley rats (Charles River, France), 10 weeks old, weighing 370 ± 4 g, were used in the present study. They were housed with a 12 h/12 h light/dark cycle (light-on from 08:00 am to 08:00 pm) and were contaminated by mineral water supplemented with either enriched (95.74% ^{238}U, 4.24% ^{235}U, 0.02%, ^{234}U, specific activity 66.3 kBq g^{-1}) or depleted uranium (99.74% ^{238}U, 0.26% ^{235}U, 0.001%, ^{234}U, specific activity 14.7 kBq g^{-1}) incorporated as nitrate at a concentration of 40 mg U l^{-1} (about 1 mg day^{-1} per rat). The control rats drank non-contaminated mineral water.

Uranium Analyses

Uranium content was measured in the kidneys, femurs, skulls, brain areas and remaining carcass. The samples were converted into ash at 600 °C, acidified and the uranium content was measured by ICP-MS (PQ Excell, Thermoelectron, France).

EEG Analyses

The EEG activity was recorded in freely moving rats by a telemetric system (Data Sciences International, USA). The transmitter was fixed intraperitoneally and the lead wires were passed under the skin to the skull where the EEG electrodes were cemented with glue and dental cement. Each implanted rat was housed with a non-implanted rat in order to avoid isolation. After a 21-day recovery period, EEG was recorded for 48 h as a control period and 48 h after exposure of 1.5 months. The data were collected and stored by an acquisition system (Somnologica software, Resmed, France). Scoring was carried out manually assigning three sleep stages (wakefulness W, slow-wave sleep SWS and paradoxical sleep PS) to 10-s periods along a time line of 24 h.

Behavioral Tests

For all behavioral tests, the rats ($n = 12$) were allowed to explore the apparatus freely. The spontaneous locomotion was measured on the first day in an open field (45 cm × 45 cm) monitored by an automated activity monitoring system (Bioseb, France). The locomotion and rearing number were recorded over 20 min. The spatial working memory was assessed on the second day in a Y-maze (three arms were 70 cm long, 50 cm high, 10 cm wide at the bottom, 20 cm wide at the top and converged at an equal angle). The sequence and number of arm entries were recorded over 10 min. Alternation was recorded if the animal entered the least recently visited arm (Pothion et al., 2004). Anxiety was assessed on the third day in an elevated plus-maze (Darnaudery et al., 2004). The number of arm entries and the time spent in each arm was recorded over 5 min.

Statistical Analyses

In all the experiments, data are expressed as mean ± S.E.M. and the effect of uranium was analyzed by ANOVA followed by Student–Newman–Keuls post hoc test. Differences were considered to be significant if $p < 0.05$.

RESULTS

Uranium accumulated mainly in the kidneys and the bones, with no significant difference between enriched and depleted uranium (Table 1). However, uranium also accumulated in some other unexpected areas such as some brain structures. In the striata, the amounts of uranium were increased by a factor of 2.2 and 2 for the rats exposed to enriched and depleted uranium, respectively, when compared to controls. More surprisingly,

Table 1
Amounts of uranium in the rat tissues after exposure of 1.5 months

Tissues	Control	Depleted U	Enriched U
Whole body	41×10^3 (5)	719×10^3 (58)*	691×10^3 (71)*
Kidneys	55 (5)	505 (69)*	453 (46)*
Adrenal	2.9 (0.4)	3.8 (0.4)	5.9 (1.0)*,#
Femurs	75 (4)	355 (32)*	307 (24)*
Skull	8.8 (1.5)	43.4 (7.4)*	38.3 (6.2)*
Whole brain	25 (1)	26 (2)	29 (1)
Cortex	7.5 (0.6)	7.1 (0.5)	6.8 (0.3)
Striatum	1.7 (0.1)	3.4 (0.8)*	3.7 (0.5)*
Hippocampus	2.0 (0.2)	2.2 (0.3)	3.9 (0.5)*,#
Hypothalamus	2.0 (0.2)	2.4 (0.4)	3.6 (0.5)*,#
Brainstem	8.9 (1.0)	6.0 (0.7)	7.4 (1.0)
Cerebellum	3.0 (0.6)	4.5 (0.7)	4.0 (0.4)

The data (expressed as nanograms of uranium (U) per tissue) are presented as means with S.E.M. in parentheses, $n = 10$.
* Different from control $p < 0.05$.
# Different from depleted uranium $p < 0.05$.

enriched uranium accumulated in some structures while depleted uranium did not. Uranium amounts were 1.5–2 times higher in the hippocampus and hypothalamus of rats exposed to enriched uranium when compared to depleted uranium rats or controls. This differential accumulation was also found in other organs such as adrenals, for which rats exposed to enriched uranium accumulated 1.5 times more uranium than did the depleted uranium rats.

After 1.5 months of exposure, electroencephalographic activity (EEG) was recorded on freely moving rats. No patent EEG abnormalities were observed while we observed sleep changes. The scoring of these EEG recordings for 24 h showed that the sleep–wake pattern was affected in rats exposed to enriched uranium, with a marked 37% increase in the amounts of paradoxical sleep (PS) from 67 ± 6 min day^{-1} to 92 ± 6 min day^{-1} that was correlated to a 26% increase in the number of PS episodes from 60 ± 3 to 76 ± 5 episodes per day (Fig. 1). In contrast, depleted uranium had no effect on the sleep architecture.

The spatial working memory capacities of the rats were assessed by spontaneous alternation examination in a Y-maze. In this test, 4% enriched uranium exposure for 1.5 months reduced the percentage of alternation from $71 \pm 2\%$ to $63 \pm 2\%$ (Fig. 2a), although depleted uranium did not. Since the decrease in alternation behavior was not associated with changes in the general exploratory activity of rats measured as a number of arm entries (Fig. 2b), it reflected a decline in the first steps of the spatial memory system.

The anxiety-like behaviour was assessed in an elevated plus-maze task. The rats exposed to enriched uranium spent 61.8% less time in the open arms (26 ± 4 s) than did the control or depleted uranium exposed rats (68 ± 13 s and 63 ± 12 s, respectively).

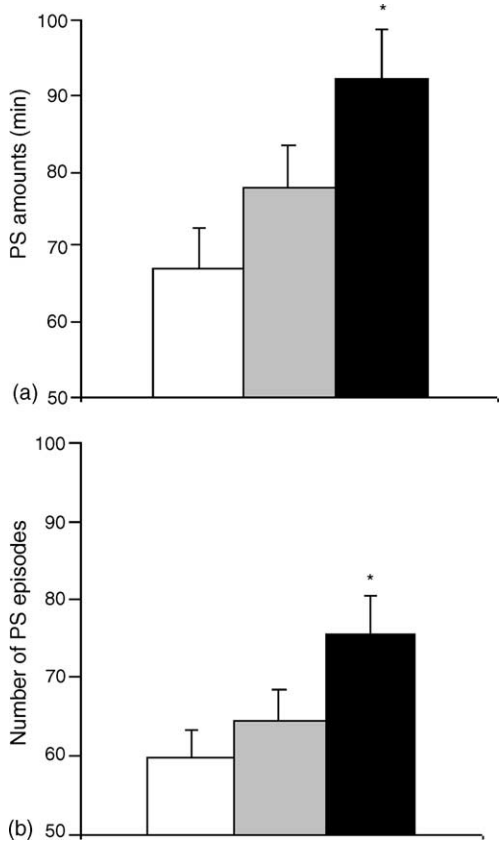

Fig. 1. Enriched uranium increased the amounts of paradoxical sleep (PS): (a) mainly by increasing the number of PS episodes and (b) of rats exposed for 1.5 months, although depleted uranium did not. The data are presented as means ± S.E.M., $n = 6$ or 8, control: white; depleted uranium: grey; 4% enriched uranium: black; *$p < 0.05$ compared to control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

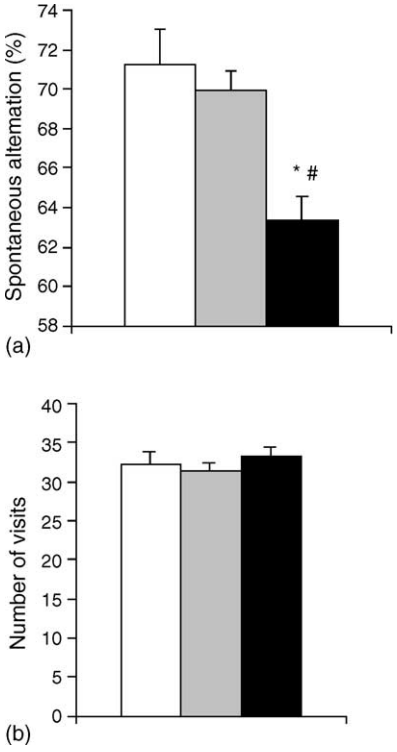

Fig. 2. Enriched uranium affected the spatial working memory of rats exposed for 1.5 months, although depleted uranium did not. The data are presented as means ± S.E.M., $n = 12$, control: white; depleted uranium: grey; 4% enriched uranium: black; * and $^{\#}p < 0.05$ compared to control and depleted uranium, respectively. The spontaneous alternation measured in the Y-maze assessed the spatial working memory (a). The number of arm entries reflects the exploratory activity in the Y-maze (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

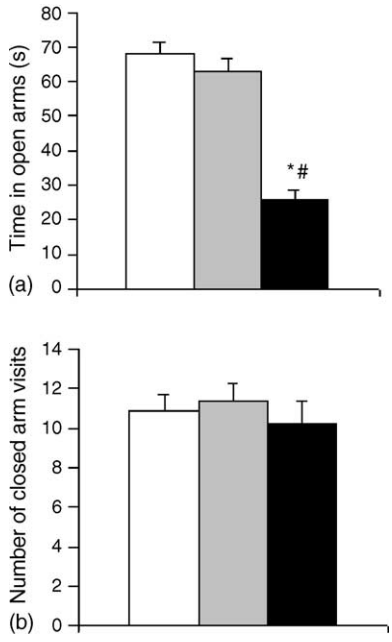

Fig. 3. Enriched uranium affected the anxiety-like behaviour of rats exposed for 1.5 months, although depleted uranium did not. The data are presented as means ± S.E.M., $n = 12$, control: white; depleted uranium: grey; 4% enriched uranium: black; * and $^{\#}p < 0.05$ compared to control and depleted uranium, respectively. The time spent in the open arms of the plus-maze assessed the anxiety level (a). The number of closed arm visits reflects the exploratory activity in the plus-maze (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

However, their general locomotor activity, measured as the number of closed arm entries, was not affected (Fig. 3a and b). This result shows that chronic exposure to enriched uranium increased anxiety-like behaviour while, once more, depleted uranium had no effect.

DISCUSSION

It is unanimously recognized that uranium accumulates in kidneys and bone. More recently, it has been shown that in some conditions of chronic exposure, uranium could enter the CNS of rats (Gilman et al., 1998; Pellmar et al., 1999a). What is remarkable here is first that after chronic exposure it accumulated in some specific brain areas and in adrenals and second that enriched and depleted uranium accumulated differently. These three new target organs concerned by enriched uranium accumulation, hippocampus, hypothalamus and adrenals, are known to be involved in the behavioral effects observed in our study. The hippocampus is known to be involved in the spatial working memory and the hypothalamic-pituitary-adrenal axis in the sleep–wake cycle and anxiety.

The neurobiological effects observed here after chronic exposure to enriched uranium could be close to those induced by chronic stress. Chronic stress was shown to induce an anxiogenic profile in the elevated plus maze task (Weiss et al., 2004) as did enriched uranium. Chronic stress was shown to induce deleterious effects on the hippocampus such as dendritic atrophy in the CA3 hippocampal region and loss of synapses between the mossy fibers and the CA3 pyramidal cells (Sousa et al., 2000). The CA3 hippocampal region plays a pivotal role in spatial learning and memory (Stepanichev et al., 2003; Wall and Messier, 2000; Lalonde, 2002; Rubaj et al., 2003). Thus, one functional consequence of chronic stress is the impairment of performance in hippocampal-dependent tasks such as spontaneous alternation examined in a Y-maze (Conrad et al., 1996, 2003; Bats et al., 2001). Exposure to enriched uranium induced a similar impairment of spontaneous alternation in the Y-maze. Chronic stress has also been shown to disturb the sleep architecture. Rats subjected to in utero stress or rats exposed to chronic mild stress spend more time in paradoxical

sleep and have an increase number of paradoxical sleep episodes (Dugovic et al., 1999; Gronli et al., 2004) like many patients suffering from depression (Van Reeth et al., 2000). Chronic exposure to enriched uranium induced similar changes in the sleep–wake cycle. We can thus supposed that enriched uranium may be acting as a chronic stressor on the central nervous system.

The mechanism by which enriched uranium induces such effects remains to be elucidated. The kidneys are unanimously considered as the most sensitive target organ to the toxicological effect of uranium. Here, the rats were healthy throughout the experimental period: their food and water intakes, body weight gain and their spontaneous locomotion measured in the open field were not affected. Moreover, the amounts of uranium measured in the kidneys of these rats were 0.12 $\mu g\ U\ g^{-1}$ kidneys i.e. far below the lowest concentration described as nephrotoxic (1.2 $\mu g\ U\ g^{-1}$ kidneys) (Diamond et al., 1989). The most probable hypothesis to explain the effects observed in the present experiment is a direct effect of uranium on one of the cerebral areas accumulating enriched uranium or on the adrenals. These effects could be chemical effects, since uranium is a heavy metal with radiological properties and some other heavy metals such as lead or methyl-mercury are known to affect the central nervous system (Nihei and Guilarte, 2001; Goulet et al., 2003). Moreover depleted uranium was shown to induce electrophysiological changes in vitro in hippocampal slices (Pellmar et al., 1999b). What remains to be determined is why enriched uranium accumulated differently from depleted uranium. Enriched and depleted uranium were introduced in the same chemical form in similar drinking water, at the same pH. This means that their chemical species were the same. The only difference between enriched and depleted uranium was their specific activities, and thus the differential accumulation of enriched uranium can only be explained by the fact that its radiological activity is four times as high. Therefore, we have evidence for an isotope effect on the deposition and distribution of uranium in various brain regions, but this pharmacokinetic difference does not argue for a pharmacodynamic difference between depleted and enriched uranium.

Our results demonstrate that chronic exposure to 4% enriched uranium, but not to depleted uranium, leads to uranium accumulation in some unexpected areas such as the hippocampus, the hypothalamus or the adrenals. Moreover, such exposure was associated with disturbances in the sleep–wake cycle, affected the spatial working memory and anxiety as early as 1.5 months after the beginning of the exposure. These effects of uranium were observed although the concentration of uranium measured in the kidneys, usually considered as the most sensitive target organ to the toxicological effect of uranium, were as non-toxic concentrations. Depleted uranium did not accumulate similarly in these areas and did not induce these effects. It will be crucial for public health to determine if depleted or natural uranium exposure could induce similar phenomena after longer periods of exposure and at different exposure levels.

ACKNOWLEDGEMENTS

We thank the following people for their advice: J.C. Bizot for the behavioural tests, V. Chazel for the alpha spectrometry, D. Clarençon for the EEG techniques, S. Frelon for the ICP-MS measurements, P. Voisin for the statistical analyses, and we thank B. Dhieux, C. Maubert, E. Tourlonias and O. Delissen for their technical help. This work is part of the Envihom program supported by the Institute for Radioprotection and Nuclear Safety.

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