FOLIA NEUROPATHOL. 1996, 34, 1
PL ISSN 0028–3894

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2-CHLORO- AND 2-BROMO-2'-DEOXYADENOSINE HAVE NO EFFECT ON THE MORPHOLOGY OF RAT NEURAL AND GLIAL CELLS IN ORGANOTYPIC CULTURE

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Organotypic cultures of hippocampus and cerebellum, established from brains of 1–3 days old rats, were exposed at different stages of development (3, 14 and 21 DIV) to 2-chloro-2'-deoxyadenosine (cladribine, 2-CdA) and 2-bromo-2'-deoxyadenosine (2-BdA) at concentrations up to 10 μM, for up to 10 days. Normal pattern and dynamics of differentiation and maturation of both neurons and glial cells was found with the use of light and electron microscopy. No ultrastructural abnormalities were induced by the substances tested. We conclude that 2-CdA and its sister compound 2-BdA do not exert cytotoxic effects toward normal rat central nervous system tissues in organotypic culture.

Key words: *brain organotypic cultures, cladribine (2-CdA), 2-bromo-2'-deoxyadenosine, cytotoxicity*

In the preceeding communication (Matyja et al. 1995) selective toxicity of a new antileukemic drug 2-chloro-2'-deoxyadenosine (cladribine, 2-CdA) and a related compound 2-bromo-2'-deoxyadenosine (2-BdA) toward the mitochondrial compartment of low-differentiated cells in organotypic cultures of human gliomas is described. Although the concentrations of 2-CdA and exposure times required to induce this mitochondrial toxicity cannot be achieved through systemic application because of bone marrow toxicity, the observation may have practical implications. Sustained, locally high concentrations of drugs at tumor site without concomitant systemic toxicity may be achieved by local infusions (Ringkjob 1968; Yamashima et al. 1990), or by implantation of a biodegradable polymer saturated with a cytotoxic compound (Brem et al. 1991).

The prerequisite for development of a local intracranial delivery of sustained and high concentrations of an antitumor drug for a clinical setting is the assessment, on animal models, of a possible neurotoxicity of the drug at high concentrations/exposure times. Since this, likewise, cannot be performed by systemic application because of non-neural toxicity, explants of normal neural tissues have been proposed as an *in vitro* approach to study neurotoxicity of cytotoxic drugs (Gilbert et al. 1989). The present experiments were perfomed to examine the effect of 2-CdA and 2-BdA upon the morphology of neurons and glial cells in organotypic culture of rat cerebellum and hippocampus during maturation and differentiation. The same concentrations and exposure times were employed, as in the previous study with organotypic glioma cultures (Matyja et al. 1995).

Material and methods

2-CdA and 2-BdA were synthesized by dr. Z. Kazimierczuk, Department of Biophysics, University of Warsaw. Tissue culture media and reagents were purchased from Sigma Chemical Co.

The experiments were performed on organotypic cultures of central nervous tissues prepared from 1–3 days old Wistar rats. Hippocampus and cerebellum were dissected out under sterile conditions and cut coronally into thin slices. The sections were put on collagen-coated glass coverslips, covered with two drops of nutrient medium and kept in Maximow double assemblies at 36.5°C. The nutrient medium consisted of 20% fetal calf serum and 80% Minimal Essential Medium supplemented with glucose to a final concentration of 600 mg%, without antibiotics. The medium was exchanged twice weekly. At different stages of development (on the 3, 14 and 21 day of growth *in vitro*, DIV) the cultures were exposed for the next 1,

3, 7 or 10 days to the medium supplemented with 2-CdA or 2-BdA at final concentration 0.3, 3 and 10 μM. Each experimental group consisted of 10 cultures and was compared with 10 control cultures grown under standard conditions. The cultures were inspected daily with a reversed light microscope. Processing of the cultures for light and electron microscopy was performed as described previously (Matyja et al. 1996).

Results

Light microscopic observation of living and fixed cultures revealed quite well preserved laminar arrangement of the hippocampal explants until the first week *in vitro*, both in control cultures and those exposed to 2-CdA or 2-BdA. The outgrowth zone of well-differentiated (21 DIV) cultures maintained in a medium supplemented with various concentration of the test agents was composed of neurons and numerous glial cells sprouting from the explants, similarily to the pattern seen in control cultures (Fig. 1a, b). Also the experimental cultures of cerebellum did not reveal any morphological changes in the explant and outgrowth zone in comparison with the sister control cultures.

Electron microscopic examination showed the intact fine structure of both hippocampus and cerebellum *in vitro* exposed to 2-CdA or 2-BdA. There were no detectable differences in dynamics of differentiation and maturation of neurons and glial cells in cultures grown for up to 10 days in a medium without and with 2-CdA or 2-BdA even at the highest concentration tested, 10 μM. Normal appearance of neurons and elements of neuropil within explants was found in all cultures exposed to the drugs.

The young (3 DIV) cultures treated with 2-CdA or 2-BdA contained immature neurons and glial cells similar to that observed in control cultures at the same stage of development. In the cultures exposed to 10 μM 2-CdA or 2-BdA several types of matured neural and glial cells could be distinguished at 14 and 21 DIV on the basis of their ultrastructural features. The hippocampal pyramidal neurons exhibited oval nuclei with dispersed chromatin. Their abundant cytoplasm contained intact cytoplasmic organellae, including numerous channels of granular endoplasmic reticulum, free ribosomes, mitochondria, well-developed Golgi complex and neurotubules (Fig. 2). The granule cells displayed a typical round or oval nucleus surrounded by a narrow rim of cytoplasm containing a few channels of granular endoplasmic reticulum, free ribosomes, small mitochondria and paucity of neurotubules (Fig. 3). The neuropil was composed of densely packed neuronal and glial processes and

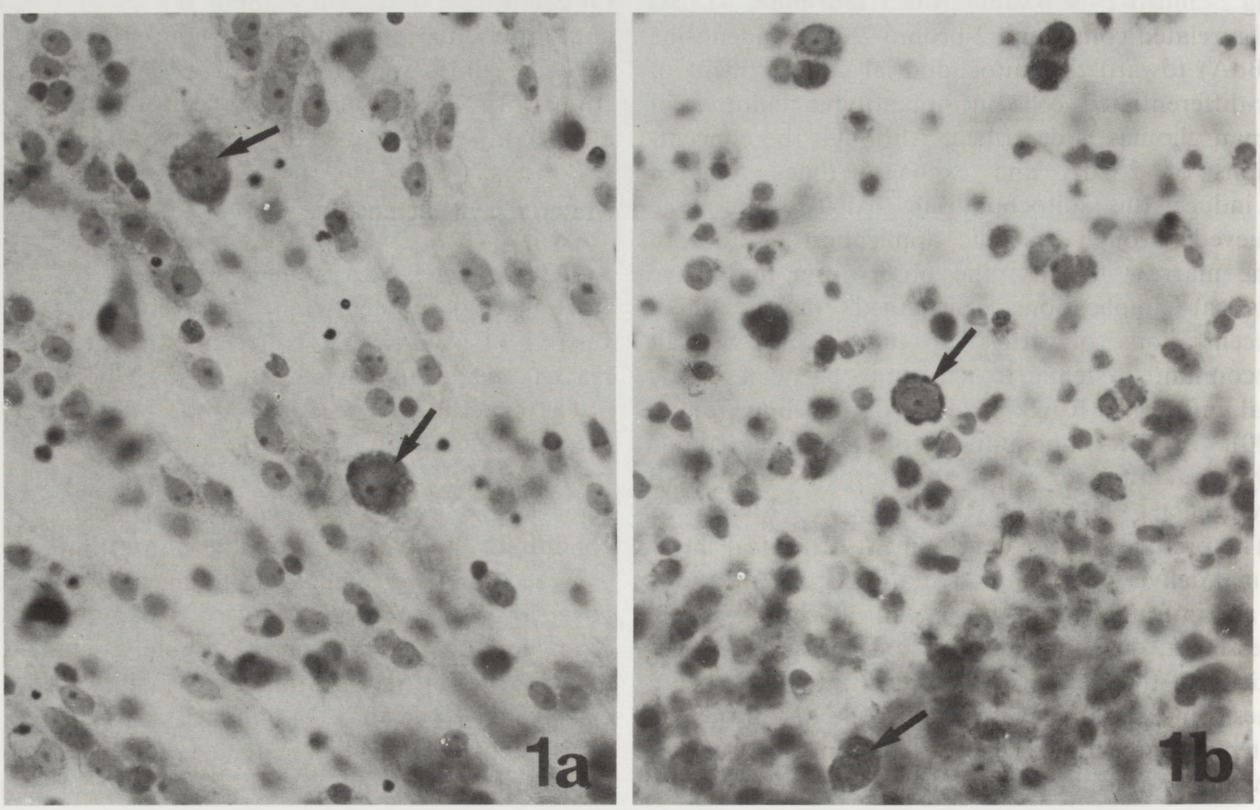

Fig. 1. a) Control culture of hippocampus (21 DIV). Numerous glial cells and a few neurons (arrows) in the outgrowth zone. ×400 b) Culture of hippocampus (21 DIV), 10 days of exposure to 10 μM 2-CdA. The outgrowth zone contains well preserved glial cells and neurons (arrows). ×400

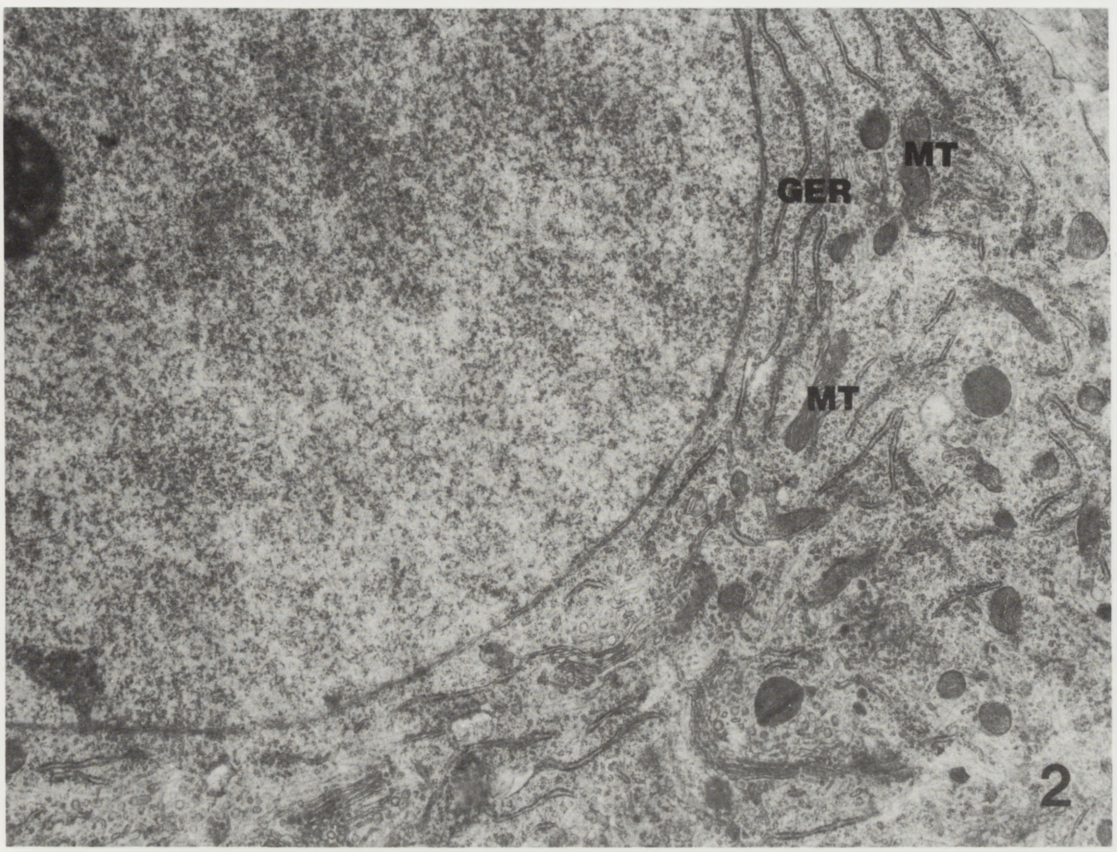

Fig. 2. Culture of hippocampus (28 DIV), 7 days of exposure to 10 μM 2-CdA. Fragment of pyramidal cell exhibited numerous well preserved mitochondria (MT), channels of granular endoplasmic reticulum (GER) and neurotubules. $\times$ 12 000

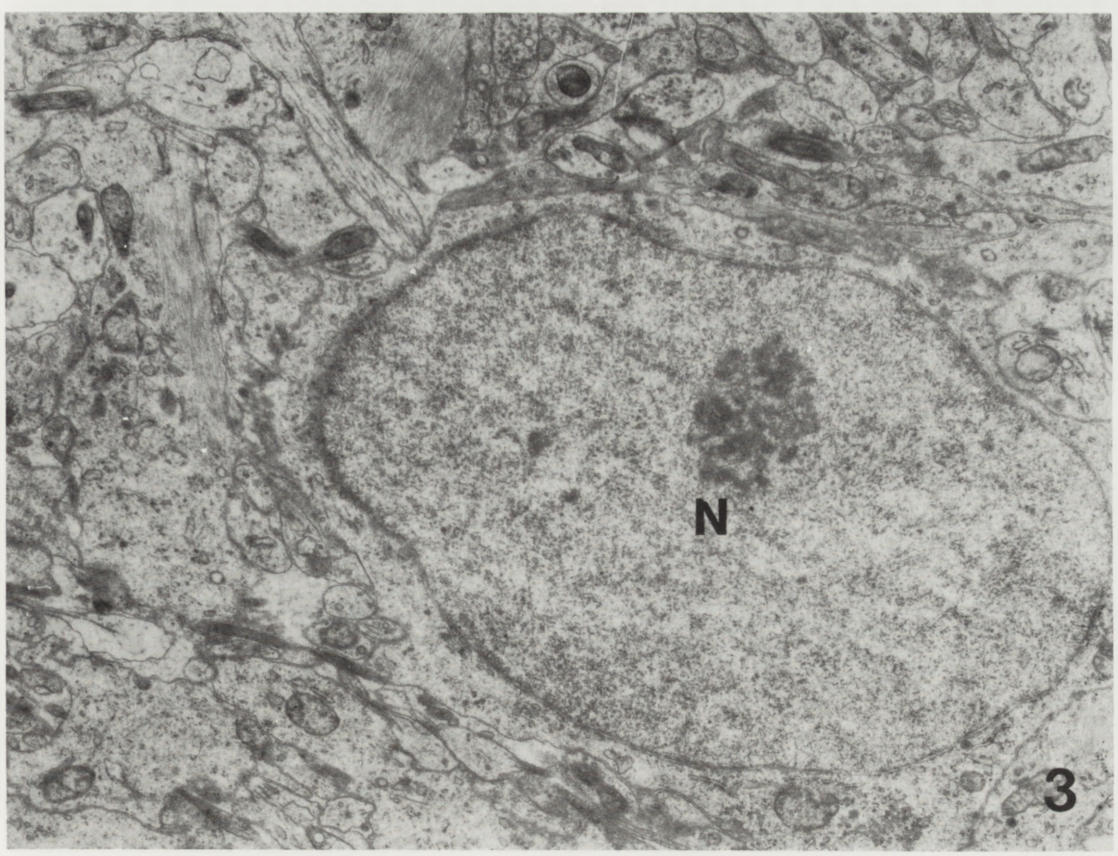

Fig. 3. The same culture. Intact granule cell (G) with round nucleus and narrow rim of cytoplasm, poor in organelles. $\times$ 10 000

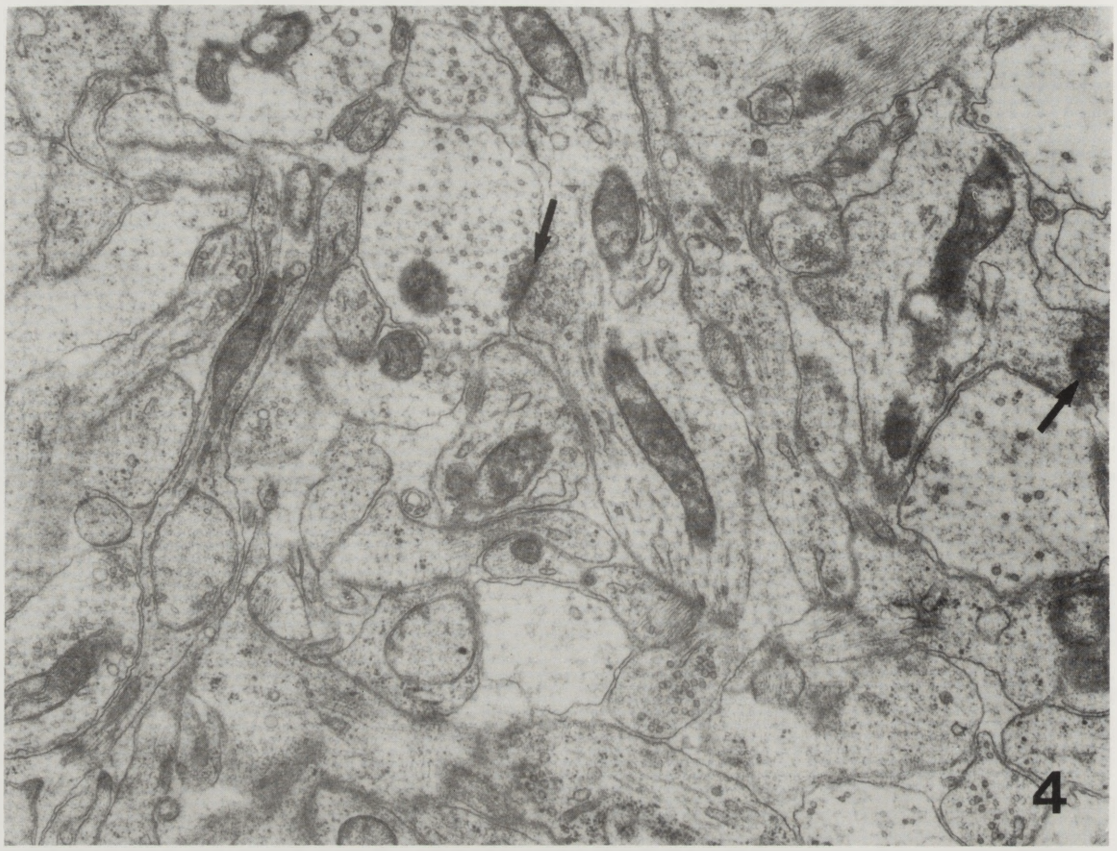

Fig. 4. The same culture. Well preserved neuropil composed of numerous neuronal and glial processes and synapses (arrows). × 20 000

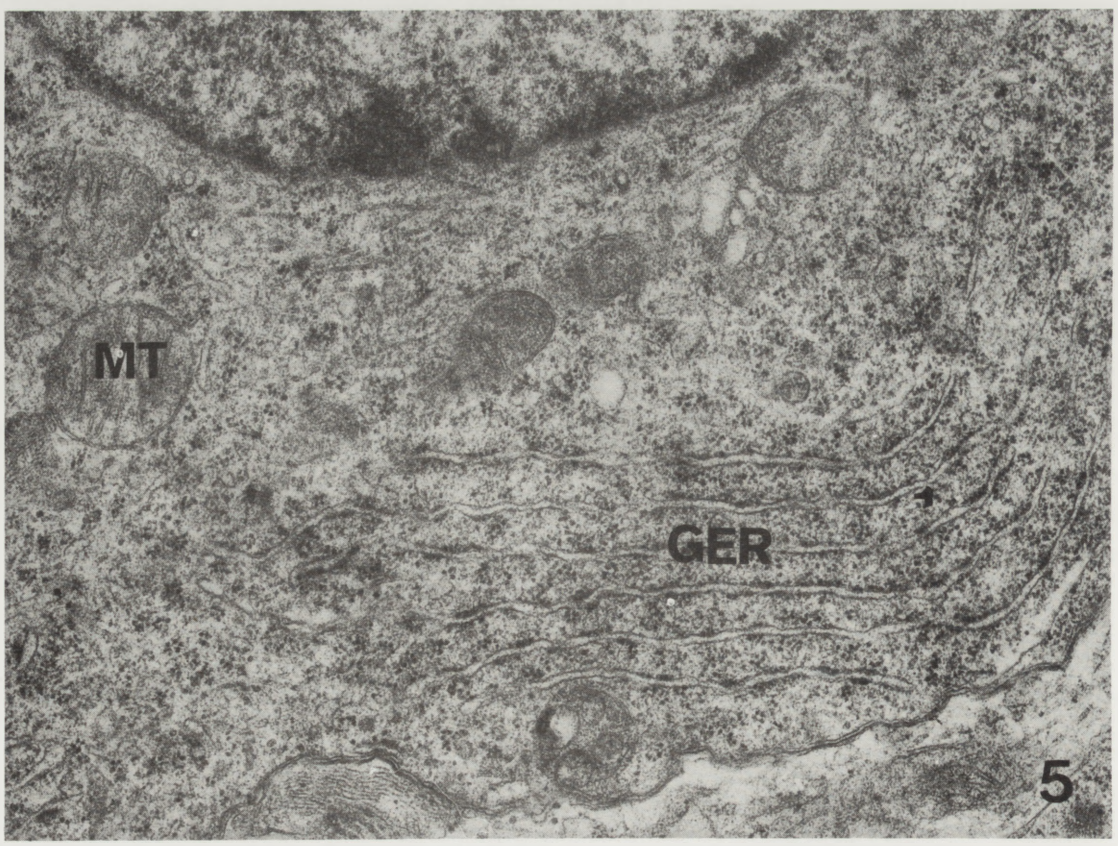

Fig. 5. Culture of cerebellum (28 DIV), 7 days of exposure to 10 μM 2-CdA. A fragment of the Purkinje cell with intact mitochondria (MT), with the parallel arrangement of granular endoplasmic reticulum (GER) and neurotubules. × 24 000

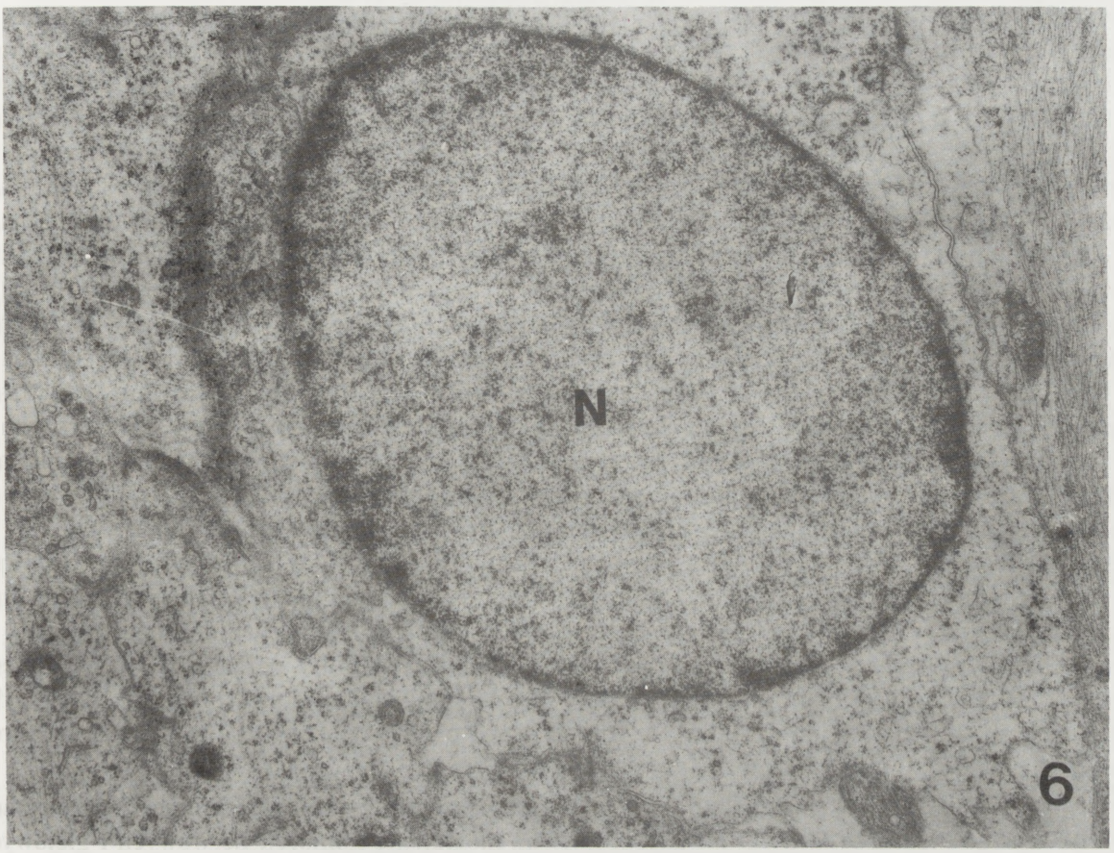

Fig. 6. Culture of hippocampus (31 DIV), 10 days of exposure to 10 μM 2-CdA. Intact protoplasmic astrocyte with round nucleus (N) and a pale cytplasm, poor in cytoorganelles. $\times$ 12 000

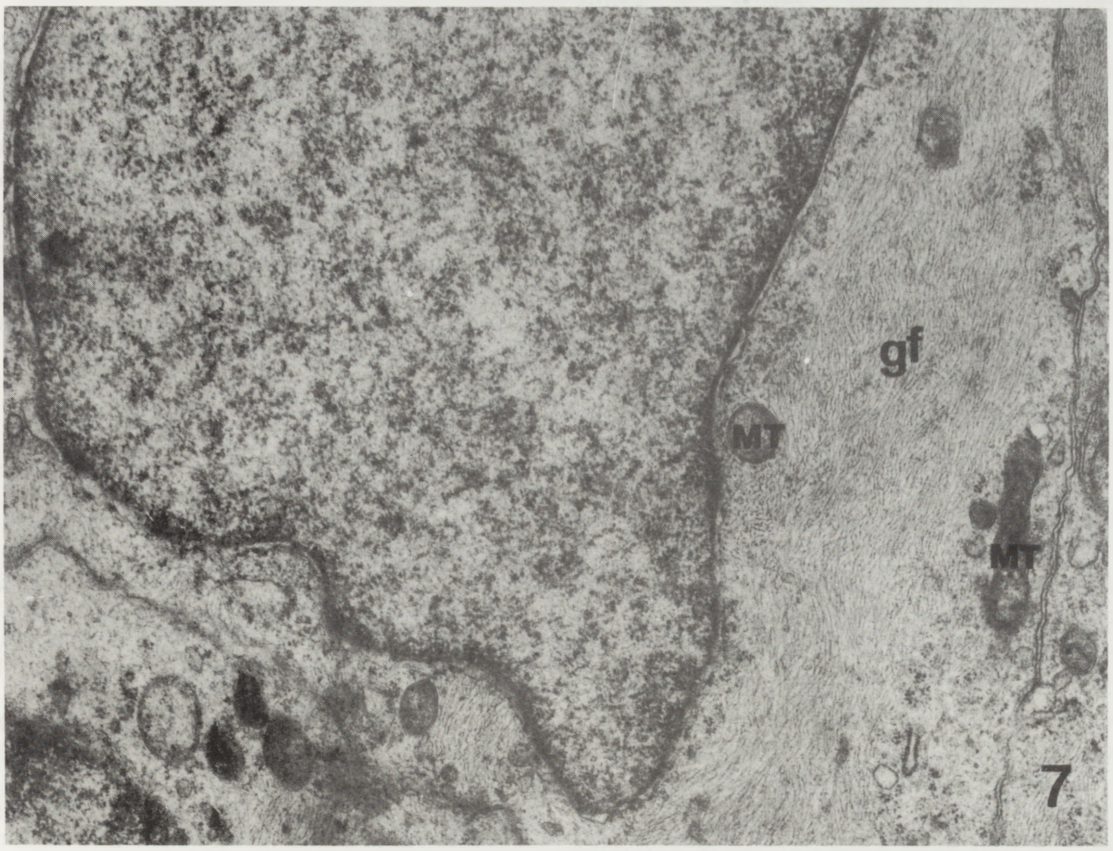

Fig. 7. Culture of hippocampus (31 DIV), 10 days of exposure to 10 μM 2-CdA. Fragment of wellpreserved fibrous astrocyte with cytopasm filled with gliofilaments (gf). A few mitochondria (MT) and short channels of granular endoplasmic reticulum. $\times$ 20 000

numerous well preserved synapses of various types, not different from the organization established in control cultures (Fig. 4). In cerebellum cultures exposed to 2-CdA or 2-BdA, the large Purkinje cells exhibited typical ultrastructural features. The prominent cytoplasm contained numerous organellae of normal appearance, including mitochondria, channels of Golgi complex, neurotubules and channels of granular endoplasmic reticulum arranged in parallel arrays of Nissl body (Fig. 5). The granule cells, usually tightly packed in clusters, showed a typical round nucleus surrounded by a thin rim of cytoplasm, poor in organelles. The glial cells in hippocampal and cerebellum cultures exposed to 2-CdA or 2-BdA faithfully corresponded in appearance to those found in control cultures. Protoplasmic astrocytes, the most common type of glia, were characterized by a round or oval nucleus and more or less abundant, electron-lucent cytoplasm, containing few organellae such as mitochondria, short cisterns of granular endoplasmic reticulum, free ribosomes and a few gliofilaments (Fig. 6). The fibrous astrocytes, numerous in outgrowth zone, revealed typical ultrastructural features. The abundant cytoplasm contained a large amount of gliofilaments, often composed in bundles. The other cytoorganelles such as mitochondria, short channels of granular endoplasmic reticulum and vesicles of Golgi complex were dispersed among the glial filaments (Fig. 7).

Discussion

Thousands of leukemia and lymphoma patients have been treated with 2-CdA (cladribine), and no central neurotoxicity of the drug has been reported following standard doses (Cheson et al. 1994). Neurotoxic effects rarely observed following doses markedly exceeding the established maximal tolerated dose were attributed to toxicity of the drug toward peripheral nerves, or seemed related to prior or concominant therapy with other drugs or irradiation (Beutler et al. 1991). Yet central neurotoxocity of 2-CdA in higher concentration/ /exposure times cannot be ruled out. Of notice is, that chemically related antileukemic drug fludarabine may exert delayed (3 to 6 weeks after treatment) central neurotoxicity with histological pattern of a progressive demyelination (Warrel et al. 1986; Kornblau et al. 1993).

In the present study no effects of 2-CdA and its sister compound 2-BdA on the growth and maturation of any cell type have been noticed in neonatal explants of rat cerebellum and hippocampus with the use of light and electron microscopy. Not only all cellular organellae appeared intact, but the cells seemed to undergo normal differentiation and maturation, including apparently undisturbed synaptogenesis. The resistance of normal rat brain tissues to the substances tested is in contrast with the mitochondrial toxicity observed in anaplastic (but not in differentiated) human glioma cells in organotypic culture (Matyja et al. 1995). In view of our present data the development of intracranial local delivery system for 2-CdA is further encouraged. It should be noted, however, that rodents, as compared to humans, are unusually resistant to the toxic effects of 2-CdA. The reason is that deoxycytidine kinase, the main enzyme activating (phosphorylating) the drug, has very different kinetic properties in humans and in rodents, resulting in 20-fold more efficient drug phosphorylation in humans ((Reichelova et al. in press). For this reason further neurotoxicity studies of 2-CdA shall be performed on primates, before firm conclusions concerning the lack of central neurotoxic properties of the drug are formulated.

Brak wpływu 2-chloro i 2-bromo-2'-dezoksyadenozyny na morfologię komórek nerwowych i glejowych szczura w hodowli organotypowej

Streszczenie

Organotypowe hodowle hipokampa i móżdżku, założone z materiału pobranego od szczurów w 1–3 dni po urodzeniu, hodowano przez 3, 10 i 14 dni. Następnie hodowle te, w różnych stadiach rozwoju, eksponowano na 2-chloro-2'-dezoksyadenozynę (cladribine, 2-CdA) i 2-bromo-2'-dezoksyadenozynę (2-BdA) w stężeniach do 10 μM przez okres do 10 dni. Metodami mikroskopii świetlnej i elektronowej stwierdzono normalny wzorzec i dynamikę różnicowania i dojrzewania komórek nerwowych i glejowych. Badane substancje nie powodowały zaburzeń ultrastruktury komórek. 2-CdA i siostrzana substancja nie wykazują efektów cytotoksycznych wobec normalnych tkanek ośrodkowego układu nerwowego szczura.

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Acknowledgements: This work was supported by grant no 0509/S4/92/03 from State Committee for Scientific Research (KBN).