ULTRASTRUCTURAL CHANGES IN VARIOUS BRAIN FORMATIONS INDUCED BY URIDINE

N. N. Karkishchenko and É. A. Bardakhch'yan

UDC 616.831-02:[615.31:547.963.3]-092.9-076.4

KEY WORDS: uridine; neurons; coated vesicles; subsurface cisterns

Some endogenous pyrmidines possess tranquilizing properties and, in addition, they may play the role of informational component for central regulation in inflammatory, degenerative, and regenerative processes [4]. The role of different parts of the brain in the mechanism of emotional reactivity, aggressiveness, and antinociception is now no longer in doubt [3, 7], although the concrete neurophysiological and, more especially, the ultrastructural changes at the single neuron level under the influence of uridine have not been studied.

The aim of this investigation was an ultrastructural analysis of certain brain formations in rats receiving uridine. Special attention was paid to coated vesicles, whose appearance is associated with specific uptake of various ligands on account of receptor-mediated endocytosis.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male albino rats weighing 180-200 g, receiving uridine in a dose of 50 mg/kg body weight intraperitoneally. In three control experiments the equivalent volume of sterile physiological saline was injected. Material for electron-microscopic investigation (pieces of the sensomotor cortex, thalamus, hippocampus, central gray matter), taken at various time intervals (30 min, 3 and 24 h) after fixation by glutaraldehyde and OsO_4, was treated by the usual method and examined in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

By contrast with other parts of the brain, administration of uridine led to the appearance of numerous coated vesicles in the capillary endothelium of the central gray matter (Fig. 1a). Moreover, sometimes all stages of endocytosis could be traced from the formation of a coated depression, and internalization to form coated vesicles. Meanwhile no ultrastructural changes, except mild microclasmatosis, were caused by uridine.

During the first half hour the first signs of activation of the energy apparatus and structures of protein synthesis could be observed in the neurons in the parts of the brain chosen for study. Many mitochrondria were swollen with a translucent matrix and with reduction of their cristae. Division of organelles was sometimes observed in the dendrites. The number of bound and free ribosomes and the number of polysomal complexes were increased. Cisterns of the rough endoplasmic reticulum showed hyperplasia. Reactive changes were taking place in the nuclei, the nucleoli were displaced toward the karyolemma, and sometimes they were increased in number to 2-3. The number of primary lysosomes also was increased. Coated vesicles and subsurface cisterns were the most interesting structure (Fig. 1b, c). Their structure, the character of the villous cover, and their function, connected with specific transport of materials and liquids [12], has not yet been finally settled. Since vesciles with an additional membrane are uncharacteristic of the cytoplasm of neurons [8], it must be assumed that they were formed in response to injection of uridine. Strictly

---

Department of Clinical Pharmacology, Faculty of Postgraduate Medicine, N. I. Pirogov Second Moscow Medical Institute. Department of Morphological Methods of Investigation, Central Research Laboratory, Rostov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 86-89, January, 1990. Original article submitted December 26, 1989.

0007-4888/90/0001-0115$12.50

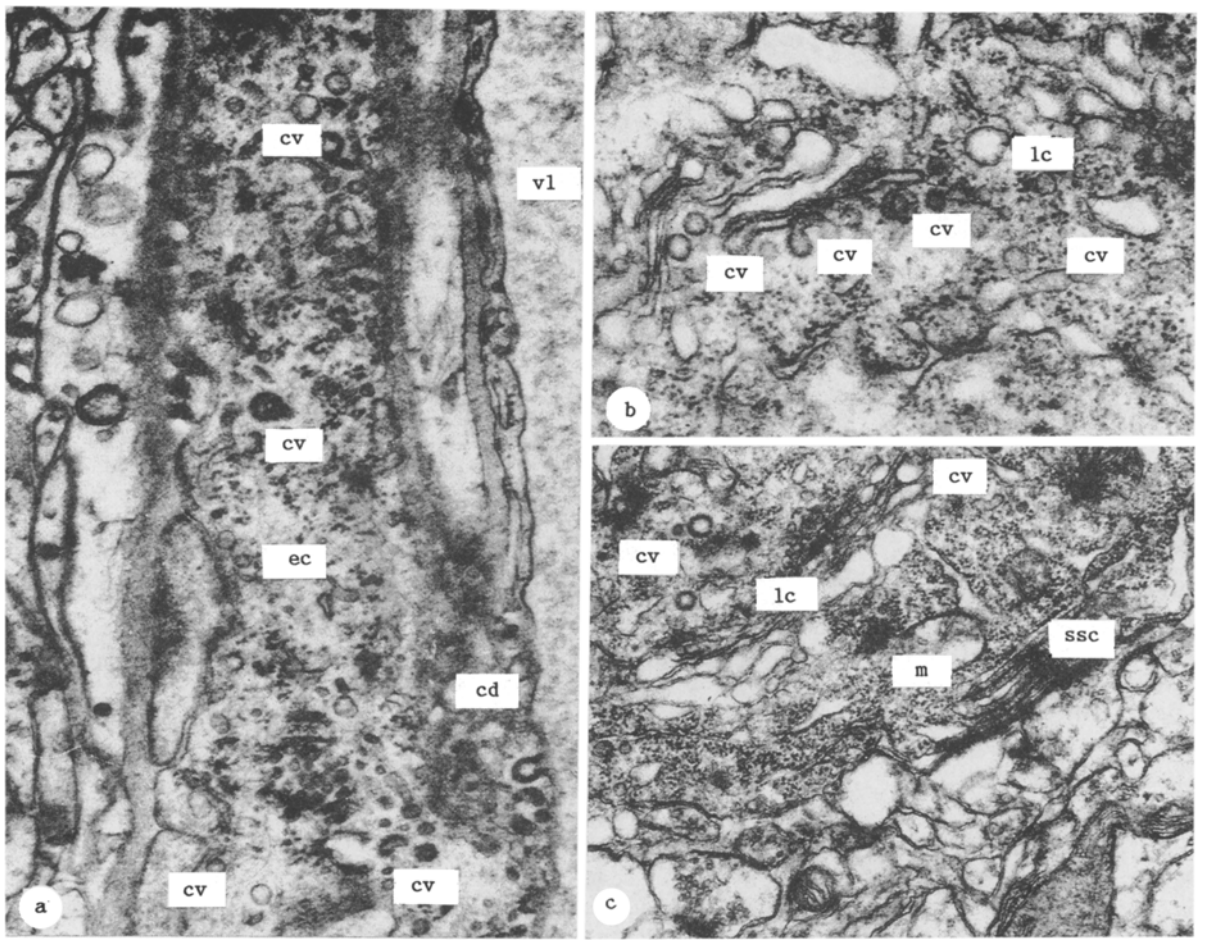

Fig. 1. Reaction of brain ultrastructures to injection of uridine. a) Formation of coated vesicles in endothelium. 41,000 ×. b) Numerous coated vesicles located in zone of lamellar complex of a nerve cell. 45,000 ×. c) Subsurface cistern (ssc). Coated vesicles of different sizes can be seen in the zone of the lamellar complex. 31,000 ×. vl) Vascular lumen, cd) coated depression, cv) coated vesicles, ec) endothelial cell, lc) lamellar complex, m) mitochrondria, n) nucleus.

controlled transfer of various molecules (polypeptide hormones, plasma proteins, asialoglycoproteins, toxins, and so on) takes place equally and has a universal mechanism [11, 13, 14]. Visualization of the coated vesicles in the endothelium and neurons in response to injection of uridine provides the basis on which to postulate interaction between its molecules and corresponding receptors on the plasma membranes of these cells.

We distinguished two types of neurons, differing in the presence or absence of receptor-mediated endocytosis. If uridine receptors were present on the plasmalemma of nerve cells, after injection of the ligand (uridine) a clear reaction, connected with passive formation of coated vesicles, which are single or absent altogether in intact animals, appeared in the cytoplasm. Both neutron populations were recorded in all parts of the CNS studied, but the level of representation of these cells without specific receptors was highest in the thalamic region.

Interaction betwen lysosomes and coated vesicles is particularly interesting. Degradation of the injection uridine evidently was carried out by the lysosomal apparatus of the cells; this reaction took place both on the plasma membrane (Fig. 2a) and in the zone of the lamellar complex (Fig. 2b). However, there also exists another pathway – nonlysosomal, connected with the participation of multivesicular bodies (Fig. 2c). Since the latter have no enzyme activity, after uptake of the coated vesicles, primary lysosomes rush toward them, and later merge with them and, after labilization of the contacting membranes, they degrade the contents of the multivesicular bodies by the corresponding enzyme systems (Fig. 2c). The final stage of intracellular processing of the internalized uridine is its degradation in lysosomes or multivesicular bodies, in agreement with data obtained by other

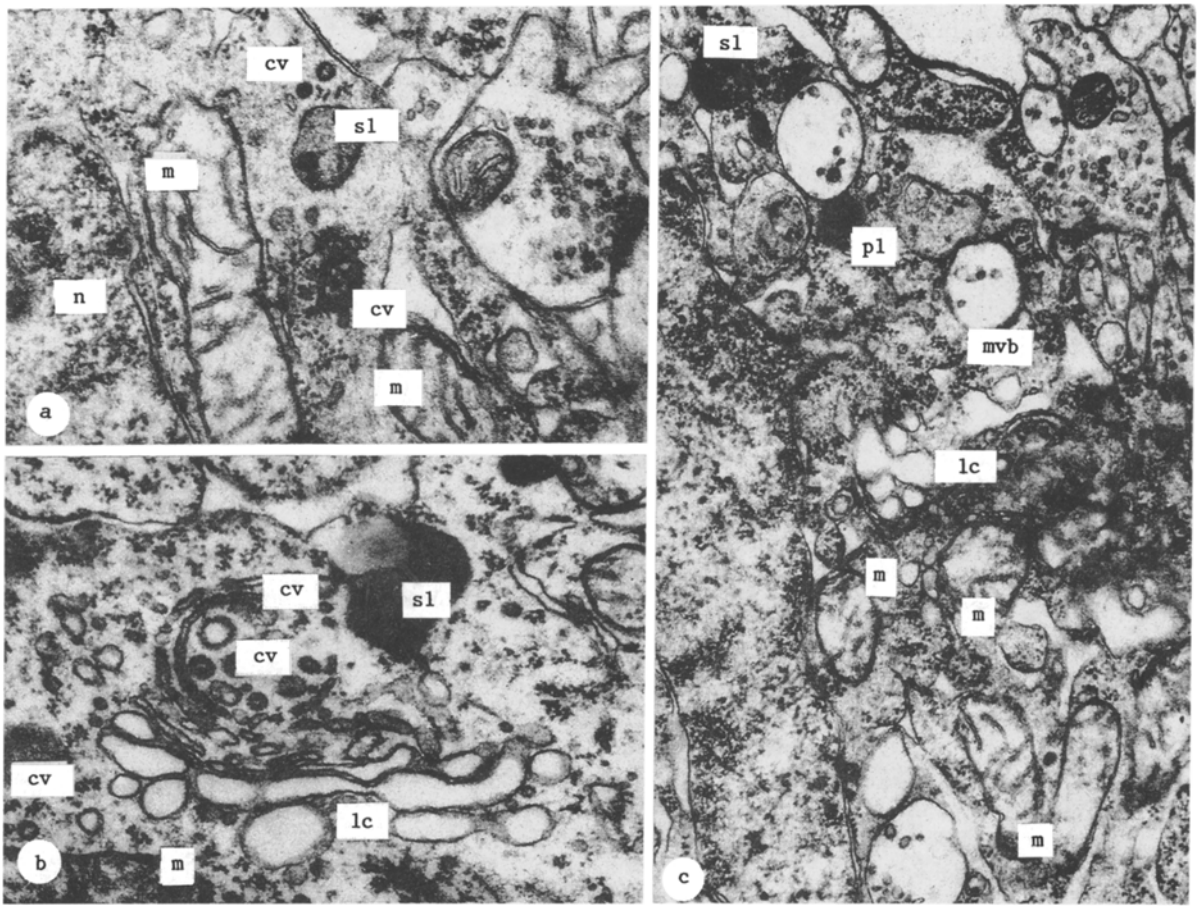

Fig. 2. Reaction of brain ultrastructures to injection of uridine. a) Secondary lysosome in immediate vicinity of coated vesicles near plasmalemma of a neuron. 45,000 ×. b) Junction between secondary lysosome and coated vesicle in zone of lamellar complex. 39,000 ×. c) Small coated vesicles in multivesicular bodies (arrows). Junction of primary lysosome and multivesicular body can be seen on the right. 32,000 ×. pl) Primary lysosome, sl) secondary lysosome, mvb) multivesicular body (remainder of legend as to Fig. 1).

workers who have studied the mechanisms of specific transport of various substances and fluids [2, 5, 9, 11, 14].

In the process of internalization of vesicles into the cytoplasm of neurons, part of the plasmalemma is naturally lost, and it may be recovered at the expense of the subsurface cistern (Fig. 1c). We observed them previously in nerve cells of the sensomotor cortex of rabbits and dogs in endotoxin shock [10], and also in rat receiving synthetic analogs of enkephalins, and we there expressed the view that their presence indicates the development of a compensatory reaction, linked with membrane deficiency and increased metabolic actdivity. Later, they are mentioned in a number of publications, whose authors, who studied the intact brain, drew attention to the more frequent localization of subsurface cisterns and mitochrondria in the zone of axodendritic junctions, and concluded that increased metabolic and, possibly, functional activity is present in these regions of the dendrites [1, 15].

The ultrastructure of a large proportion of interneuronal junctions was undisturbed in the paths of the brain studied with the exception of the hippocampus, where occasionally destruction in synapses was observed, of the pale and even of the dark type.

The most important result of this investigation is thus the possibility of identifying two types of neurons, differing in their ability to form coated vesicles in response to administration of uridine. The presence or absence of uridine receptors on the plasma membrane of nerve cells may be reponsible for the complex functional mosaic of neuronal activity and differences in the responses of nerve cells to this ligand. So far as the fate of the

coated vesicles is concerned, as specialized organelles they transport the ligand to the lysosomes, where it is degraded. The appearance of subsurface cisterns is a compensatory reaction to a deficiency of the neurolemma, partner in the neuron–gliocyte metabolic system that is most resistant to the action of uridine.

LITERATURE CITED

1. V. P. Babmindra, Arkh. Anat., No. 10, 5 (1983).
2. É. A. Bardakhch'yan, P. E. Povilaitite, and A. I. Polyak, Byull. Éksp. Biol. Med., No. 6, 730 (1988).
3. A. V. Val'dman, and Yu. D. Igantov, The Central Mechanisms of Pain [in Russian], Leningrad (1976).
4. N. N. Karkishchenko, Regulation of Information and Regeneration in Surgery [in Russian], Rostov-on-Don (1976), pp. 45-50.
5. N. N. Karkishchenko, V. V. Solodilov, and É. A. Bardakhch'yan, Izv. Sev. Kavkaz. Nauch. Tsent. Vyssh. Shkoly, No. 2, 122 (1987).
6. N. N. Karkishchenko, V. V. Solodilov, and É. A. Bardakhch'yan, Izv. Sev. Kavkaz. Nauch. Tsent. Vyssh. Shkoly, No.1, 121 (1988).
7. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
8. G. V. Morozov and N. N. Bogolepov, Morphinism [in Russian], Moscow (1984).
9. P. E. Povilaitite, É. A. Bardakhch'yan, and G. A. Vilkov, Tsitol. Genet., No. 1, 78 (1986).
10. K. I. Polyanin and É. A. Bardakhch'yan, Arkh. Anat., No. 7, 5 (1980).
11. M. S. Brown and J. L. Goldstein, Natl. Cancer Inst. Monogr., 60, 3 (1982).
12. J. L. Goldstein, R. G. W. Anderson, and M. S. Brown, Nature, 279, 679 (1979).
13. O. W. Petersen and B. van Deurs, J. Cell Biol., 96, 266 (1983).
14. C. F. Simmons and A. L. Schwartz, J. Cell Biol., 99, 373 (1984).
15. R. K. Space, J. Neurocytol., 11, 693 (1982).