Effect of Morphine on the Number and Branching of Astrocytes in Various Regions of Rat Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 3, pp. 297-299, March, 2001
Original article submitted October 31, 2000

We studied the effects of morphine in high doses on astrocytes in the nucleus accumbens, lateral septum, and caudate nucleus of rat brain. Activation of astrocytes in the nucleus accumbens and lateral septal nucleus was manifested in hyperplasia and elongation of astrocyte processes. In the caudate nucleus, the total length of astrocyte processes and branching of individual astrocytes decreased.

Key Words: *rat; brain; astrocytes; morphine; immunocytochemistry*

Microglial cells and astrocytes activated during various pathological processes in the central nervous system (CNS) produce neurotoxic cytokines (*e.g.*, interleukin-1β), free oxygen radicals, and NO. These agents cause neuronal degeneration and secondary activation and dedifferentiation of astrocytes accompanied by inhibition of astrocyte functions (uptake of glutamate, K^+, and Ca^{2+} from the intercellular space, release of neurotropic factors, in particular, nerve growth factor). These changes intensify pathological processes and increase the count of degenerated neurons [6-9].

Exogenous opiates modulate the structural and functional organization of the neuroglia. Morphine in a dose of 10^{-4} M inhibits astrocyte growth in primary culture of mouse brain. In a dose of 10^{-8}-10^{-10} M, morphine decreases proliferative activity and differentiation of astrocytes, increases the cytoplasmic volume, and causes hypertrophy of cell processes. [3, 10,11].

The effects of endo- and exogenous opioids on the neuroglia are realized via opioid receptors on plasma membranes of neuroglial cells. Receptors for opiate alkaloids (Mu3 receptors) were found in primary culture of astrocytes and microglial cells from cat brain. Moreover, morphine antagonist naloxone attenuates the effect of morphine on the neuroglia [3,8,10,11]. However, it was reported that morphine even in high concentrations does not affect viability of astrocytes [4].

Here we studied the effects of morphine in high doses on astrocytes in the nucleus accumbens (NA, *n. accumbens septi*), caudate nucleus (CN, *n. caudatus*) and lateral septum nuclei (LSN, *n. lateralis septi*) [2, 13]. These brain structures are most sensitive to morphine and participate in the formation of psychic and physical drug dependence.

MATERIALS AND METHODS

Experiments were performed on outbred albino rats weighing 230-250 g. The rats were daily intraperitoneally injected with 2 ml 1% morphine for 9 days. Control animals received an equivalent volume of physiological saline.

On day 10 of the experiment, the animals were narcotized with chloral hydrate and the brain was perfused with 4% glutaraldehyde in phosphate buffer (pH 7.4). Fixed brain was placed in the same solution containing 30% sucrose. For identification of astrocytes, 40-μ frontal slices were treated for 12-14 h with antiglial fibrillary acidic protein (GFAP) monoclonal antibodies, and treated with biotinylated second antibodies and acridine-biotin-peroxidase complex. Immune complexes were visualized with 3,3-diaminobenzidine tetrachloride (0.005%) and H_2O_2. Highly specific immunocytochemical assay reveals only astrocytes and their processes.

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0007-4888/03/1313-0248$25.00

Astrocyte count (N) and the total length of astrocyte processes (L) per section area were calculated by the formula [15]:

$$L=\pi/2\times I\times d/M,$$

where I is the number of intersections of astrocyte processes with morphometric grid lines, d is the distance (μ) between lines of the morphometric grid, and M is the final magnification of microphotography. The degree of astrocyte branching was calculated as L/N.

Quantitative measurements were performed using a morphometric grid with parallel lines (5-mm spaces). The final magnification of microphotographs was 440, and the section area was 93,600 μ^2 (arbitrary area unit). The calculations were performed by the formula: $L=17.8\times I$. We examined 15-20 microphotographs of each sample.

The results were analyzed by Student's t test.

RESULTS

In control animals, the mean number of astrocytes in CN differed from that in NA and LSN (Fig. 1, *a*), which confirmed that NA is phylogenetically closer to the septum than to CN [12].

The total length of astrocyte processes in CN surpassed that in LSN (Fig. 1, *b*).

Branching of individual astrocytes in the examined structures considerably varied (Fig. 1, *c*). The total length of astrocyte processes and their branching indicated close phylogenetic similarity between NA and LSN.

Morphine had no effect on the total number of astrocytes in brain structures (Fig. 1, *a*). The total length of astrocyte processes decreased by 17.5% in CN ($p<0.05$), but in LSN and NA this parameter increased by 24 ($p<0.01$) and 17% (insignificant, Fig. 1, *b*), respectively. The degree of cell branching decreased by 22.69% in CN ($p<0.01$), but increased by 39.4 ($p<0.01$) and 34% ($p<0.01$, Fig. 1, *c*) in NA and LSN, respectively.

We found no significant differences in the number of astrocytes between the control and morphine-treated rats, which was probably related to the facts that morphine does not cause astrocyte death, and brain astrocytes in adult rats possess low proliferative activity (in adult animals the number of H^3-thymidine-labeled (proliferating) cells was only 0.08% of the to-

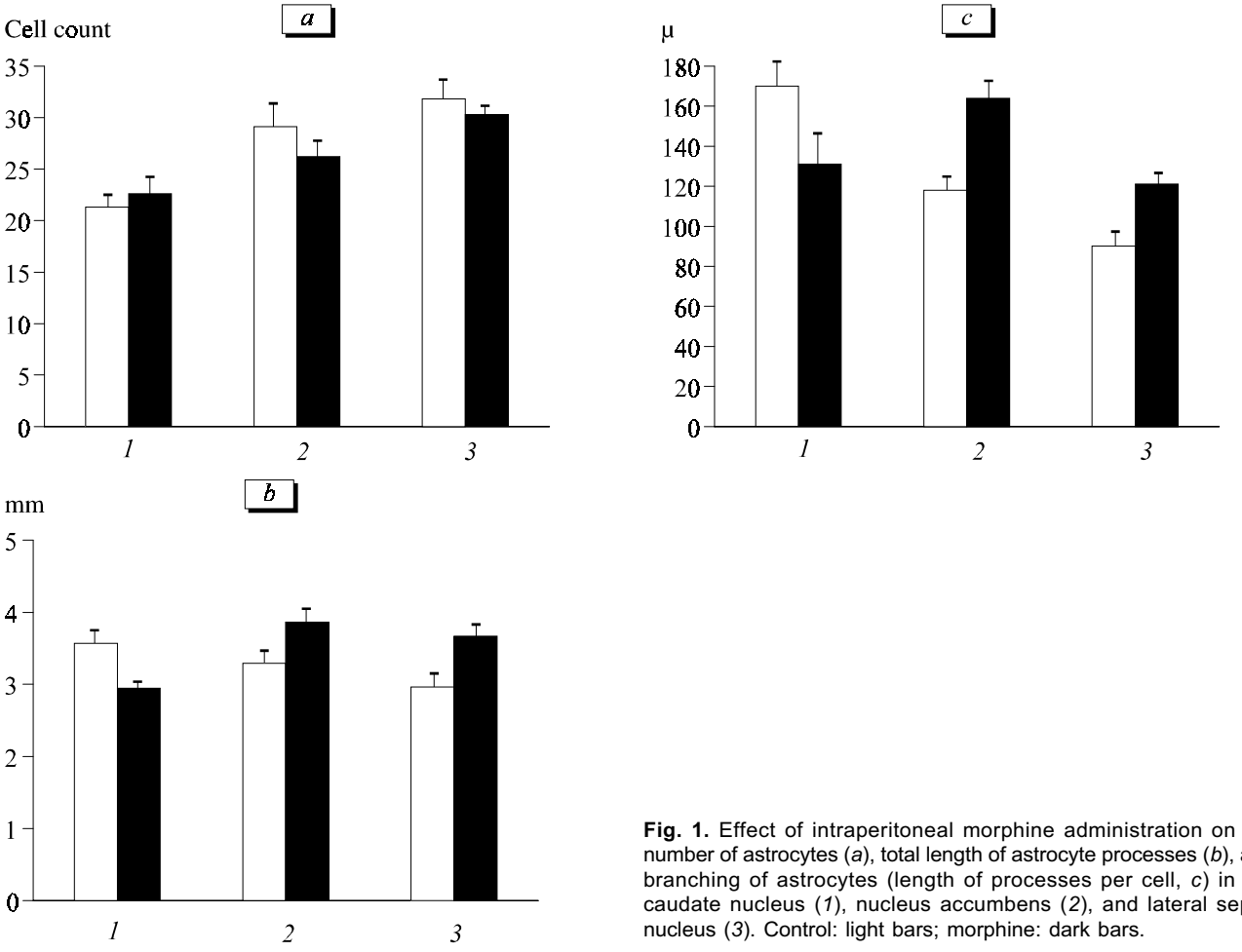

Fig. 1. Effect of intraperitoneal morphine administration on the number of astrocytes (*a*), total length of astrocyte processes (*b*), and branching of astrocytes (length of processes per cell, *c*) in the caudate nucleus (*1*), nucleus accumbens (*2*), and lateral septal nucleus (*3*). Control: light bars; morphine: dark bars.

tal astrocyte population [5,14]). Therefore, it is difficult to study the effect of morphine on astrocyte proliferation in adult animals.

Our findings indicate that astrocytes in these brain structures are characterized by different sensitivity to morphine. Morphine induces hyperplasia of astrocytes in NA and LSN, which is manifested in elongation of astrocyte processes. However, morphine inhibits activity of astrocytes in CN and decreases the total length of their processes and branching. This immunocytochemical assay reveals relatively large astrocyte processes containing gliofibril bundles. Previous studies showed that threshold nociceptive stimulation of dental pulp, enucleation of the eyeball, and social isolation of animals lead to pronounced hyperplasia of not only large, but also lamellar processes of astrocytes in the CNS [1]. These data suggest that not only fibril-containing, but also thin lamellar astrocyte processes in NA and LSN undergo hyperplasia.

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