Histo- and Cytoarchitectonics of the Hippocampus in Young Rats Injected with Propofol and Mexidol

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 4, pp. 457-459, April, 2010
Original article submitted December 18, 2009

Light microscopic examination of the hippocampus was carried out in young rats (intact and injected with propofol and mexidol according to different protocols). Propofol had a negative effect on hippocampal neurons causing an almost 2-fold increase in the count of modified cells in comparison with the normal level. Mexidol effectively corrected these shifts.

Key Words: *hippocampus; propofol; mexidol; morphology*

Propofol is often used in medical practice. It is a drug from list B, a preparation for noninhalation narcosis characterized by rapid (after 30-60 sec) and short-lasting effect. On the other hand, it is known that many drugs with such effects can have an unfavorable impact for CNS neurons. This problem is particularly important for childhood. The development and use of neuroprotectors is needed to detect and reduce these consequences. A prospective drug is mexidol, characterized by antihypoxic, neuroprotective, antioxidant, and antistress effects.

We studied the effects of propofol and propofol+mexidol in the hippocampus of young rats. The hippocampus was selected as the object of the study because it rapidly and actively (in comparison with other brain structures) reacts to treatment of this kind and is one of the main objects used for screening of bioactive substances.

We evaluated the effect of total intraperitoneal anesthesia on the developing brain and the efficiency of neuroprotective mexidol therapy.

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MATERIALS AND METHODS

The study was carried out on outbred young male rats (60 g) kept on standard rations in accordance with regulations on handling laboratory animals. The animals were divided at random into 5 groups (1 control and 4 experimental), 5 per group. Experimental animals were subjected to total intraperitoneal anesthesia with propofol in a dose of 20 mg/kg, the duration of narcosis was 30 min. Group 1 animals received propofol, group 2 received two injections of mexidol intramuscularly in a dose of 150 μg/kg 24 h and 30 min before anesthesia. Group 3 rats received two injections of mexidol intramuscularly in a dose of 150 μg/kg 24 h and 30 min before anesthesia and then in the same dose daily for 3 days. Group 4 rats were injected with mexidol in the same dose intramuscularly after anesthesia, then daily for 3 days in the same dose. Hence, group 2 rats received mexidol before narcosis, group 3 animals before and after narcosis, and group 4 animals received mexidol only after narcosis.

The material for studies was collected from all animals on day 3 after propofol injection. After sacrifice, the brain was removed, the hippocampal zone was isolated, fragments of the brain were fixed in 10% neutral formalin, processed routinely, and embedded

0007-4888/10/1494-0471

in paraffin. Serial sections (6-8 μ) were sliced from paraffin blocks and mounted on slides treated with a mixture of egg albumin and glycerol.

The sections were stained by Nissl method and with hematoxylin and eosin.

Morphological changes in field CA1 (subfields a, b, c), CA2, CA3 (subfields a, b, c), and CA4 hippocampal pyramidal neurons were quantitatively evaluated in the preparations stained after Niessle from animals of all groups by the method developed by A. V. Svishchev for quantitative evaluation of neuronal status in the CNS organs [2]. A table was composed and a special formula was used. The parameters and arbitrary notions essential for the use of this formula are presented in Table 1.

The degree of morphological changes in CNS formation was evaluated by the formula:

$$DC=\frac{(2b+3c+4d+5e+5f+6g+6g+6f)}{a+2b+3c+4d+5e+5f+6g+6h}\times 100\%,$$

where DC is the degree of changes in neuronal status, a, b, c...h denote the numbers of neurons of the corresponding groups (Table 1). The cells were counted in 5 visual fields of 1 serial section at magnification 10×10 and expressed in percents. The results of morphological studies were statistically processed using parametric Student's test.

The preparations stained with hematoxylin and eosin were used to evaluate the total count of neuronal population and the count of dead neurons by computer-aided morphometric analysis.

RESULTS

Neuronal population of the hippocampal pyramidal layer was primarily presented by unchanged cells. The intensity of staining of the nucleus and components of the basophilic substance attested to active functioning of the majority of cells characteristic of the developing brain. Along with intact neurons, modified cells were detected, with the status corresponding to groups II-V (Table 1). The content of small hyperchromatic neurons (group V) was 4-6%. They were most often seen in the CA3 field (about 10%), and also in CA1, CA2, CA4 fields (2-3%). Total content of modified cells was 18% of the entire neuronal population.

In group 1, the total content of modified neurons in all fields of view 2-fold surpassed the normal. The detected signs of disorders in their structure corresponded to the states specified for groups II-VI (Table 1). Initial swelling and chromatolysis (group II) were detected in the majority (58%) of modified cells; 18% were in a state corresponding to group IV, 21% in a state corresponding to group III. Solitary neurons had sharply vacuolated cytoplasm (group VI).

The neuronal population in group 2 was in general close to normal. Hyperchromatic cells were detected (no more than 12%; group V; Table 1). About 28% were cells with initial swelling (group IV).

The neuronal population in group 3 was also close to the normal one. No more than 10% neurons were hyperchromatic and up to 30% of the total count of modified cells were group II neurons.

The neuronal population in group 4 was also in general close to normal. The status of the majority of modified neurons corresponded to groups II-V (Table 1); few hyperchromatic cells with shrunk perikaryon were seen.

The study revealed no coarse changes in neurons in the reference (intact) group or in experimental groups. The detected changes were reversible.

The results of statistical analysis of the counts of modified hippocampal neurons in different experimental groups are presented in Figure 1.

TABLE 1. Quantitative Evaluation of Neuronal Status (Score)

Group (neuronal status)	Group	Neuronal status (score)	Neuronal counts in groups
Normal neuron	I	1	a
Initial swelling and tigrolysis	II	2	b
Pronounced swelling and tigrolysis	III	3	c
Swollen neuron with hyperchromatosis	IV	4	d
Dehydrated hyperchromatic neuron	V	5	e
Vacuolated neuron	VI	5	f
Atrophic neuron	VII	6	g
*Dead neuron		6	h

Note. *No neurons of this kind were detected, and hence, this group was not included in estimations.

The number of modified neurons in the hippocampus increases significantly (more than 2-fold) after narcosis. Mexidol treatment (groups 2, 3, 4) significantly reduced this value.

The counts of modified neurons in groups 2 and 3 were significantly lower than in group 1 and virtually did not differ from the normal level. The mean count of modified neurons in group 4 also virtually did not differ from the normal, though with a trend to an increase in their levels in comparison with groups 2 and 3 (before anesthesia or before and after anesthesia). This fact can be regarded as a lower protective effect of mexidol in comparison with other protocols of its use.

Coincidence of the mean levels of modified neurons in groups 2 and 3 can be attributed to long half-life period (more than 4 h) of mexidol. This drug, injected for the last time 30 min before narcosis, still acted during several hours after narcosis. Therefore, the protocols of mexidol treatment in groups 2 and 3 differed only by the drug dose persisting in the body 30 min after narcosis. In group 3 this dose was 1.5-fold higher than in group 2. This presumably explains a lesser range of values in group 3 (virtually coinciding with the range in the reference group) compared to group 2.

According to computer morphometry of the preparations stained with hematoxylin and eosin, the neuronal population counts were virtually the same in the reference group and in all experimental groups. These data coincide with the results obtained on the preparations stained after Nissl for various hippocampal fields (CA1, CA2, CA3, CA4).

The results indicate a negative effect of propofol on hippocampal neurons of young rats, however, causing no irreversible changes. Mexidol is characterized by a pronounced neuroprotective effect and decreases the counts of damaged neurons. The effect of mexidol was somewhat more pronounced, when it was administered before (or before and after) narcosis.

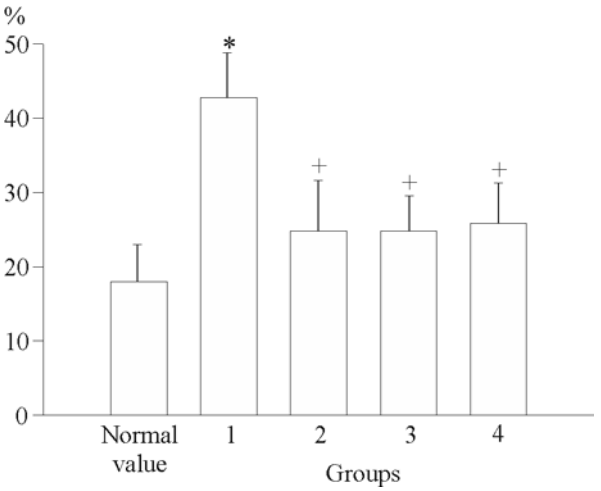

Fig. 1. Percentage of modified hippocampal neurons in intact and experimental rats. *p<0.001 compared to normal level, ^{+}p<0.01 compared to group 1.

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