Morphochemical Study of Hippocampus of August Rats after Systemic Treatment with Amphetamine Followed by Injection of Delta Sleep-Inducing Peptide

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Quantitative interferometry showed that chronic amphetamine administration to August rats (2.5 mg/kg/day for 3 weeks) increased the area of neuronal cytoplasm and nuclei and content and concentrations of proteins in hippocampal CA3 neurons. These changes persisted after single injection of delta sleep-inducing peptide (60 μg/kg). The reaction of the entire neuronal population of hippocampal CA3 neurons to amphetamine is similar.

Key Words: *August rats; hippocampus; amphetamine; delta sleep-inducing peptide; neuron interferometry*

August rats are predisposed to mental stress and study of their hippocampus, the brain structure playing a leading role in the organization of emotional reactions, motivations, and memory, attracts special interest, particularly in the amphetamine model of dopaminergic system hyperfunction. This condition is described as schizophrenia-like state leading to behavioral disorders in animals [4]. It seems that delta sleep-inducing peptide (DSIP) modulating brain metabolism and electrical activity by increasing the spectral power of delta rhythms [9] can be used for the correction of some pathological processes in mammalian nervous system, including those associated with hyperactivity of the dopaminergic system. Liability to mental stress is associated with DSIP insufficiency in animal brain [7]. However, the effect of this peptide on morphochemical changes in hippocampal neurons of animals with amphetamine-induced dysfunction of the dopaminergic system is little studied.

We studied morphochemical parameters (size of the cytoplasm and nuclei of neurons and protein content and concentrations in these cells) in the hippocampus of August rats after a single systemic injection of DSIP against the backgroud of long-term amphetamine treatment.

MATERIALS AND METHODS

Experiments were carried out on August rats (160-180 g). The animals were divided into 3 groups. Controls were injected with saline. Animals of experimental group 1 receiving chronic amphetamine treatment (intraperitoneally, 2.5 mg/kg/day for 3 weeks) were sacrificed 30-40 min after the last injection. Rats of experimental group 2, injected with DSIP (intraperitoneally, 60 μg/kg) after chronic amphetamine treatment, were sacrificed 30-40 min after the last injection. The rats were decapitated under light ether narcosis. The brain was fixed in Carnoy fluid and processed histologically by the protocol used in our laboratory [3]. The brain was embedded in paraffin and 7-μ sections were made on a microtome.

Dry weight of compact substances in the cytoplasm and nuclei of hippocampal CA3 neurons was determined by interferometry. This parameter reflects the content and concentration of structural

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proteins in the neurons in fixed material [1] (a test reflecting protein function). Profile fields of the neuronal nuclei and cytoplasm were measured by MOB-1-15 ocular micrometer. Sections stained with Cresyl Violet [2] served as morphological control.

RESULTS

In August rats receiving amphetamine the area of nuclei and cytoplasm of hippocampal CA3 neurons increased by 13 and 51%, respectively. Protein content in neuronal cytoplasm and nuclei increased by 121 and 89%, respectively; protein concentration increased by 51 and 66%, respectively. Distribution curves of the studied values demonstrate regularity and similar reaction to amphetamine for the entire population of neurons in the hippocampal CA3 field (Fig. 1).

Hence, chronic amphetamine treatment significantly increased the area of CA3 neurons and the

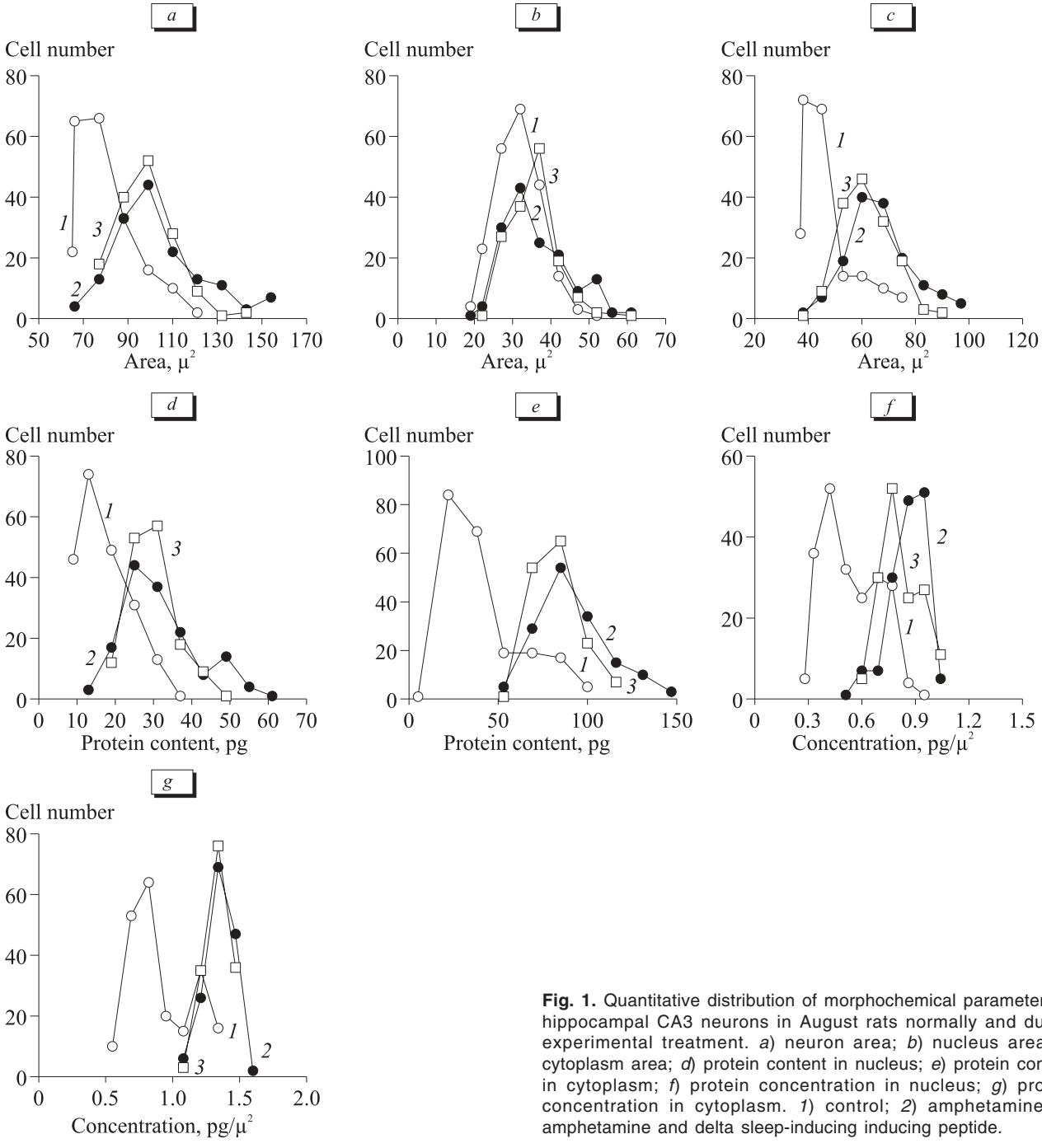

Fig. 1. Quantitative distribution of morphochemical parameters of hippocampal CA3 neurons in August rats normally and during experimental treatment. *a*) neuron area; *b*) nucleus area; *c*) cytoplasm area; *d*) protein content in nucleus; *e*) protein content in cytoplasm; *f*) protein concentration in nucleus; *g*) protein concentration in cytoplasm. *1*) control; *2*) amphetamine; *3*) amphetamine and delta sleep-inducing inducing peptide.

content and concentrations of protein substances in their cytoplasm and nuclei. The reaction of the neuronal cytoplasm to amphetamine was more pronounced. Functional activity of these neurons seems to correlate with their protein content.

Clinical picture of amphetamine psychosis in humans is considered to be similar to that of acute or chronic schizophrenia. A characteristic sign of these conditions is stereotypy, detected in model experiments with amphetamine. In Wistar rats these conditions are associated with EEG desynchronization in different structures of the brain, presenting as the so-called "amphetamine" waves [6].

A single injection of DSIP after amphetamine treatment did not modify the parameters of hippocampal neurons of August rats, which virtually did not differ from the effect of amphetamine alone. The curves of distribution of the parameters indicated retained similar reactions of all neurons in the hippocampal CA3 field after subsequent injection of DSIP (Fig. 1).

In Wistar rats resistant to mental stress DSIP gradually restored motor activity modified by amphetamine. On the other hand, EEG of brain structures showed persisting amphetamine waves, starting to be periodically interrupted by slow waves [6].

In August rats, amphetamine treatment stimulates function of hippocampal CA3 field neurons (all studied parameters increase). This state seems to be stable in August rats predisposed to mental stress, because subsequent injection of DSIP virtually does not modify these parameters. On the whole, the size of neuronal nuclei and cytoplasm and protein content and concentrations in these cells, which we regard, similarly as other authors [5], as the integral indicator of the status of structural proteins during training, not only precisely shows their role in integrative activity of the brain, but also demonstrates the function of some brain structures in these processes.

Lower level of delta sleep-inducing peptide in the brain of August rats compared to Wistar rats more resistant to mental stress was previously reported [7]. There are data on preventive effect of DSIP in experimental stress [8-10]. Resistance of modified neurons of the hippocampal CA3 field to DSIP is demonstrated on the model of amphetamine-induced dopaminergic dysfunction in August rats, used in this study.

It seems that the absence of normalizing effect of DSIP can be attributed to the fact that August rats predisposed to stress need either other doses of the peptide or other scheme of its administration (for example, preventive or repeated). The present results prompt that the relationship between the initial behavioral characteristics of animals and morphochemical features of their brain structures, specifically, limbic characteristics, should be taken into consideration.

REFERENCES

1. V. Ya. Brodskii, *Cell Trophics* [in Russian], Moscow (1966).
2. I. V. Viktorov, *Modern Methods for Morphological Studies* [in Russian], Moscow (1966), pp. 7-10.
3. L. M. Gershtein, *Tsitologiya*, **21**, No. 4, 424-428 (1979).
4. V. G. Kolpakov, *Catatonia in Animals* [in Russian], Novosibirsk (1990).
5. V. N. Mats and O. L. Segal, *Neurochemical Bases of Training and Memory* [in Russian], Moscow (1989), pp. 135-155.
6. N. S. Popova and E. L. Dovedova, *Ros. Fiziol. Zh.*, **84**, Nos. 1-2, 24-29 (1998).
7. R. M. Salieva, E. V. Koplik, Z. A. Kamenov, and A. B. Poletaev, *Byull. Eksp. Biol. Med.*, No. 10, 464-466 (1989).
8. K. V. Sudakov, *Zh. Vyssh. Nervn. Deyat.*, **37**, No. 1, 78-87 (1987).
9. K. V. Sudakov, *Individual Resistance to Mental Stress* [in Russian], Ryazan (1998).
10. E. M. Khvatova, N. A. Rubanova, I. A. Prudchenko, and I. I. Mikhaleva, *Progress in Functional Neurochemistry* [in Russian], St. Petersburg (2003), pp. 120-126.