PHARMACOLOGY AND TOXICOLOGY

Regional and Subcellular Localization of Cycloprolylglycine in Rat Brain

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HPLC analysis showed that endogenous cyclopeptide cycloprolylglycine exhibiting mnemotropic and anxiolytic properties is nonuniformly distributed between brain structures in rats: its contents in the whole brain, cortex, and hippocampus were 29.2±1.6, 38.9±8.0, 126.4±32.4 nmol/g, respectively. Cycloprolylglycine distribution between subcellular fractions of brain neurons is also nonuniform: 60% cyclopeptide appeared in the nuclear fraction.

Key Words: *cycloprolylglycine; regional and subcellular localization; biologically active cyclic peptides*

Endogenous cycloprolylglycine (CPG) was first detected in rat brain by HPLC and chromatography-mass spectrometry [2]. Evaluation of pharmacological properties of CPG showed that it exhibits mnemotropic activity in doses of 0.1-1.0 mg/kg intraperitoneally. CPG facilitates information input, but impedes memory trace retrieval in passive avoidance test in rats; it improves learning in active avoidance paradigm in rats and positively affects interhemispheric information transfer by increasing the magnitude of transcallosal evoked potentials in rats [4,6]. In addition, CPG possessed *in vitro* anticoagulant and fibrinolytic properties in concentrations of 10^{-5}-10^{-10} M and modulates membrane potential in synaptoneurosomes in concentration 10^{-6} M [5,7]. In experiments on rats, CPG exhibits anxiolytic activity in elevated plus maze test in doses of 0.05-0.10 mg/kg intraperitoneally [3] and in open field test in doses of 0.01-0.10 mg/kg [8]. Anxiolytic effect of CPG is selective. It manifests in linear animals with pronounced fear reaction and is absent in animals with active type of behavior. CPG content was found to depend on the type of emotional stress reaction of the animals. CPG content in the whole brain of stress-resistant C57Bl/6 mice was 1.5 times higher than in non-stress-resistant Balb/c mice [8]. This suggests that CPG plays a role of endogenous anxiolytic agent.

Heretofore, both subcellular CPG localization in neurons and its distribution between brain structures were unknown. This study is dedicated to fill this gap. Since CPG possesses mnemotropic and anxiolytic properties, we estimated CPG concentrations in the cerebral cortex and hippocampus, the structures involved in learning and memory processes and in anxiety reaction [11]. Peculiarities of subcellular localization of CPG can be a clue to deciphering its mechanism of action.

MATERIALS AND METHODS

Experiments were carried on male outbred albino rats (n=15) weighing 220-250 g and obtained from Stolbo-

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TABLE 1. Content of CPG and Other Neuropeptides in Brain Structures of Rodents

Peptide	Peptide content per 1 g wet tissue		
	whole brain	hippocampus	cortex
CPG, nmol	29.2±1.6 (*n*=4)	126.4±32.4 (*n*=8)*	38.9±8.0 (*n*=4)
Substance P [10], pmol	–	4.08±0.61	6.98±0.98
Cholecystokinin-4 [11], pmol	–	20±8	33±5
Neuropeptide Y [12], pmol	–	22±6	19±2

Note. *Hippocampi were united in pairs for extraction, in all other cases one extraction corresponded to one animal. All cases included 3 parallel chromatographic estimations.

vaya nursery. The animals were kept in the vivarium of V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences at 12-h light-dark cycle and on standard ration. All manipulations were performed in cold. The animals were decapitated; the brain was immediately removed, washed with cooled physiological saline, dried, and then processed similarly for all objects. The following preparations were used in the study: whole brain, hippocampus, and brain cortex. They were homogenized in a glass homogenizer for 1 min in ice-cold distilled water (1:3). Homogenates of the whole brain were centrifuged at 3000 rpm for 15 min, non-purified nuclear fraction of the brain tissue and supernatant were obtained, the latter was centrifuged at 12,000 rpm for 30 min to obtain mitochondrial fraction and supernatant containing microsomal fraction (synaptosomes). All three fractions (nuclei, mitochondria, and synaptosomes) and homogenates of the whole brain, cortex, and hippocampus were extracted with a 3-fold volume of acetonitrile for 10 min on an electric shaker. Acetonitrile extracts were evaporated to dryness in a rotor evaporator. Dry residue was stored at -20°C. Then, the residues were dissolved in 1 ml eluent and chromatographic evaluation was performed as described before [1]. CPG content was calculated using the absolute calibration approach. The results were processed statistically (Statistica 6.0).

RESULTS

CPG content in structures of rat brain differed significantly (Table 1). CPG concentration in the cortex and hippocampus surpassed its mean concentration in the brain by 1.3 and 4 times, respectively. Higher CPG level in the hippocampus probably plays an important physiological role in antistress and cognitive processes. It should be noted, that another mnemotropic and anxiolytic peptide (neuropeptide Y) exhibits similar distribution in rat brain: it is preferentially localized in the cortex, hippocampus, and striatum [12,13]. Such mnemotropic and anxiogenic peptides as substance P [9] and cholecystokinin-4 [14] were found in the cortex and hippocampus.

TABLE 2. CPG Distribution in Subcellular Fractions of Rat Brain Tissue

CPG content, μg per brain (*n*=3)		
nuclei	mitochondria	synaptosomes
5.13±0.35	1.55±0.17	1.86±0.25

Note. *n*: number of animals; three parallel estimations were performed in each animal.

CPG distribution in subcellular fractions of rat brain tissues is presented in Table 2.

These data suggest that almost 60% CPG is localized in nuclear fraction of rat brain, whereas its content in synaptosomes and mitochondrial fraction is significantly lower: 22% in synaptosomal fraction and 18% in mitochondria fraction.

These findings can be important for elucidation of the role of intracellular localization of bioactive CPG with nootropic and anxiolytic properties in the realization of its antistress and nootropic pharmacological effects. High CPG level in the nuclei may indicate its interaction with nuclear, but not membrane receptors. There are data for non-classical nuclear localization of certain regulatory peptides and their receptors, such as Met(5) enkephaline [15] and insulin [10].

Thus, the content of biologically active CPG with nootropic and anxiolytic properties in brain structures and its intracellular localization were investigated. Its non-uniform distribution in brain structures and intracellular localization in rat brain can be related to manifestations of its biological effects.

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