ACTION OF DELTA SLEEP INDUCING PEPTIDE ON MONOAMINE OXIDASE AND ACETYLCHOLINESTERASE ACTIVITY IN SUBCELLULAR FRACTIONS FROM RABBIT BRAIN FORMATIONS *in vivo*

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UDC 612.822.1.015.1:577.152.143]-06:616.822.1

KEY WORDS: delta sleep inducing peptide; motor system of the brain; fractions of synaptosomes and mitochondria; monoamine oxidases of types A and B; acetylcholinesterase.

To study the central mechanisms of motor responses a promising method is to examine the principles governing metabolism of different parts of the brain belonging to the motor system at the cellular and subcellular levels under the influence of neuropeptides with a sedative or activating action [9, 11]. One such peptide is delta sleep inducing peptide (DSIP). Its action has been studied on various processes linked with mechanisms of sleep and also on some physiological and chemical reactions [2, 4]. Previously, a study of the action of DSIP *in vitro* in concentrations of 1×10^{-6}-1×10^{-5} revealed activation of type A (substrate — serotonin) monoamine oxidase (MAO) in subcellular fractions of synaptosomes and intracellular mitochondria from rabbit brain, whereas activity of type B (substrate — p-nitrophenylethylamine) MAO and of acetylcholinesterase (AChE) was unchanged [1].

The aim of the present investigation was to study the effect of DSIP (synthesized in the Shemyakin Institute of Bioorganic Chemistry [8]) on parameters of activity of the same enzyme systems at the subcellular level in formations of the rabbit motor system *in vivo*.

EXPERIMENTAL METHOD

DSIP was injected by the suboccipital route into rabbits in a dose of 30 μg/kg body weight in 0.05 ml physiological saline. Cortical and subcortical formations of the motor (sensory-motor cortex, caudate nucleus, and ventrolateral thalamic nuclei) were investigated. Subfractions of synaptic membranes, light and heavy synaptosomes, and mitochondria were isolated from bodies of neurons for biochemical study by differential and gradient centrifugation of these formations. In the subfractions (1-5 μg protein/ml sample) spectrophotometric estimation of protein was carried out by the method in [7], of AChE activity as in [6], type A MAO activity (substrate — serotonin) as in [10], and type B activity (substrate — p-nitrophenylethylamine) as in [3].

EXPERIMENTAL RESULTS

Under the influence of DSIP, 30 min after suboccipital injection of the peptide the specific activity of AChE and of MAO of types A and B was substantially altered in fractions of membrane synaptosomes and intracellular mitochondria compared with the control (Fig. 1). The action of DSIP on different aspects of mediator metabolism was quite selective both for individual enzyme systems and for the different brain formations and subcellular components.

AChE activity as a whole showed little change under the influence of DSIP, but it was reduced by 20-25% in light synaptosomes and membranes of the sensory-motor cortex and, in particular, in membrane fractions from the thalamus.

Type B MAO activity was significantly reduced in intracellular mitochondria of the sensory-motor cortex (by 50%) and caudate nucleus; it was also reduced considerably in some fractions of synaptosomes.

Laboratory of Histobiochemistry, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A.P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 56-58, May, 1982. Original article submitted November 17, 1981.

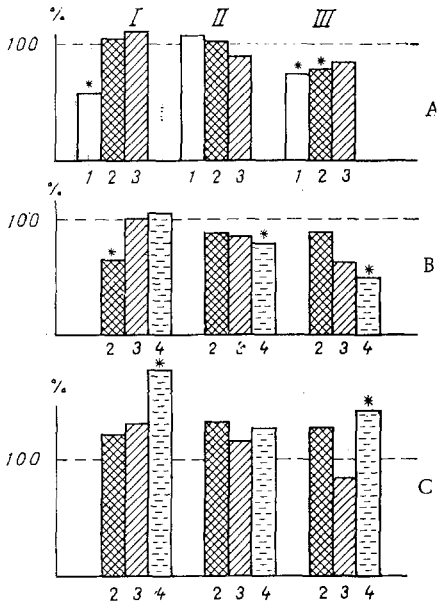

Fig. 1. Effect of DSIP on enzyme activity in subcellular fractions of different brain formations. A) AChE, B) type B MAO, C) type A MAO. I) Thalamus, II) caudate nucleus, III) sensory-motor cortex. 1-4) Subfractions of synaptic membranes, light synaptosomes, heavy synaptosomes, and free intracellular mitochondria, respectively. Ordinate, enzyme activity (in %).

Type A MAO activity, on the other hand, was increased; this was most marked in the thalamus and, in particular, in intracellular mitochondria in this formation (by 69%). Activity of type A MAO also was increased in subfractions of mitochondria from the sensory-motor cortex (by 41%).

The general trend of changes in the activity of type A MAO *in vivo* thus corresponds to the changes observed previously *in vitro* [1]. However, DSIP had demonstrable activity on type B MAO and AChE activity only *in vivo*.

Administration of DSIP *in vivo* under these experimental conditions thus affected mediator (catecholamine and acetylcholine) metabolism in motor structures of the brain. Comparison of these findings with recent reports of the physiological effects of DSIP [5] indicates that DSIP exerts regulatory functions. Their connection with mechanisms of sleep is not yet clear. The possibility cannot be ruled out that the role of DSIP in these processes is not its only, and perhaps not its chief function. As regards the serotoninergic system, the fact that changes in type A MAO were in the same direction *in vivo* and *in vitro* suggests a direct role of DSIP in reactions of serotonin utilization. This influence is seen most clearly in fractions of mitochondria in the neuron body. The fact that changes were more marked in free mitochondria than in mitochondria of synaptosomes can be attributed to the greater accessibility of the substrate for enzyme action.

The decrease in AChE activity, manifested particularly in subfractions of cortical membranes and light synaptosomes may lead to a rise in the acetylcholine level in response to injection of DSIP *in vivo*. It can be tentatively suggested that this effect is indirect, for no effect on AChE was observed *in vitro*. The same remarks also apply to the inhibitory action of DSIP *in vivo* on type B MAO activity, which was unchanged *in vitro*.

The similar effects of DSIP on type A MAO activity *in vivo* and *in vitro* thus allow the molecular objects of the direct action of this peptide to be identified, and the changes, which are manifested only *in vivo*, are probably indirect effects.

The authors are grateful to Corresponding Member of the Academy of Medical Sciences of the USSR Professor V. T. Ivanov for supplying the preparation of delta sleep inducing peptide used in the work.

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ENZYMES DETOXICATING ACTIVE FORMS OF OXYGEN AND LIPID PEROXIDES IN EXPERIMENTAL MYOCARDIAL ISCHEMIA AND INFARCTION

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UDC 616.127-005.4+616.127-005.8]-092.9-07:616.127-008.931:577.152.2]-074

KEY WORDS: myocardial ischemia and infarction; superoxide dismutase; glutathione peroxidase; glutathione-S-transferase.

Recent experimental data have confirmed the important role of lipid peroxidation (LPO) in the pathogenesis of ischemic heart disease [5, 8]. Activation of LPO has been found during the formation of a myocardial infarct [4]; some natural and synthetic antioxidants have been found to inhibit the development of necrosis [4, 11]. Antioxidant enzymes — superoxide dismutase (SOD) and glutathione peroxidase (GP) — responsible for detoxication of the superoxide O_2 anion-radical and for decomposition of lipid peroxides [6], take part in the regulation of LPO *in vivo*. According to some workers [13, 15], a definite role in the utilization of lipid peroxides in the tissues may be played by certain glutathione-S-transferases (GTF), although this view has not been fully substantiated [9, 14]. The present writers showed previously that acute ischemia of the liver leads to a sharp fall in SOD and GP activity; a fall in the activity of antioxidant enzymes, moreover, may be one of the main causes of the intensification of LPO in ischemia [1].

The object of this investigation was to study changes in activity of SOD, GP, and GTF in the zone of ischemia and necrosis during the development of an infarct following coronary occlusion in rats.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. I. M. Sechenov First Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 58-60, May, 1982. Original article submitted November 27, 1981.

0007-4888/82/9305-0613$07.50