Effects of delta-sleep inducing peptide (DSIP) and some analogues on the activity of monoamine oxidase type A in rat brain under hypoxia stress

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Abstract Metabolic effects of delta-sleep inducing peptide (DSIP) under hypoxia stress were investigated in rats subjected to short-term hypoxic conditions (about 0.26 Bar). It was found that DSIP partially restricted stress-induced changes in activity of mitochondrial monoamine oxidase type A (MAO-A) and serotonin level in rat brain. A number of DSIP analogues was tested and among them there were some compounds with enhanced ability to counteract hypoxia induced changes in MAO-A activity and serotonin content in comparison with native neuropeptide.

Key words: DSIP and analogues; Hypoxia; Brain mitochondrial MAO type A; Serotonin

1. Introduction

Humoral sleep factor DSIP (delta-sleep inducing peptide) was isolated from cerebral venous blood of rabbits and its structure was determined by Swiss research group (M. Monnier and G. Schoenenberger, 1977). This linear endogenous nonapeptide received the name delta-sleep inducing peptide due to its ability to increase EEG slow wave sleep (or delta-sleep) in rabbits [1]. Somnogenic activity of synthetic DSIP was reported in a number of papers in various animal species and in human but some other publications did not confirm somnogenic properties (for a review see [2]). Although Swiss authors consider DSIP as a natural regulator of sleep-waking function [2] hypnogenic action of DSIP still remains doubtful.

DSIP has a number of other nonspecific for sleep effects [3]. According to our data the most pronounced feature of its multifunctional physiological action is the highly expressed stress-protective and adaptogenic activity. Thus, DSIP after peripheral administration in a small dose (100–200 μg/kg) significantly increases resistance of animals to acute emotional stress by prevention of cardiovascular disturbances [4,5], enhances adaptation to cold by normalization of cold-induced biochemical shifts [6,7], prevents metabolic changes in brain under experimental hypoxia [8], inhibits metastatic spreading and remarkably increases the stability of organism to tumour growth due to restoration of neurohumoral indices and the immunomodulating potency of the host [9,10]. DSIP has also well documented antiepileptic activity under experimental application of different seizure inducing agents [11]. A number of DSIP analogues was synthesized and their antiepileptic and antimetastatic potency were investigated in some test-models along with DSIP. Certain structure–activity dependences were established for this series of compounds [9,12].

2. Materials and methods

The experiments were carried out on white not throughbred male rats (180–200 g). Hypoxia stress was modelled by pressure chamber under 0.26 Bar (that corresponds to altitude of 10 km above the ground level) during 15 min. Peptides were dissolved in saline and injected intraperitoneally in a dose of 120 μg/kg 20 min prior to stressfull manipulations.

The mitochondria and cytoplasmic fractions were isolated from rat brain as described in [13]. Mitochondria were then purified by differential centrifugation in the gradient of saccharose density (0.32; 0.80 and 1.2 M). Monoamine oxidase activity was determined by means of isothermic ammonia evaporation using saturable concentration of serotonin as substrate by modified Gorkin method [14]. The content of serotonin in rat brain was measured by procedure described in [15].

DSIP and analogues were synthesized as described in [12] by solid-phase method on the 9500 Peptide Synthesizer (MiliGen/Biosearch). Final products were obtained by gel-filtration on Sephadex G-15 or preparative RP HPLC, the content of desirable substances was more than 97%. The purity and individuality of the peptides was controlled by HPLC, FAB-mass spectrometry, NMR-spectroscopy and amino acid analysis.

For data analysis, Student's *t*-test was used.

3. Results and discussion

Changes in enzyme activity are a key mechanism for regulation of metabolic processes under alterations in functional state of an organism. Monoamine oxidase (MAO) is known as an important brain enzyme participating in metabolism of biogenic monoamines. It determines significantly their level in the brain and thereby provides a control of their neurotransmitter and hormonal functions. MAO ranks among membrane enzymes and is located essentially on the exterior membrane of mitochondria. Its activity depends on a lipid environment. The regulative role of membrane lipids upon properties of MAO was established [16,17]. There are two types of MAO in brain and other tissues: MAO-A and MAO-B. MAO-A has its own specific substrates of oxidation: serotonin and noradrenaline. This enzyme is contained essentially in monoaminoergic neurons and acts intraneuronally while MAO-B has extraneuronal localization in glial cells [16].

Investigation of MAO activity in brain of rats subjected to action of stressful stimuli revealed significant changes in its activity in the case of cranium-brain trauma [18], hyperoxia [19] and barometric hypoxia [8]. Earlier Krichevskaya et al. [7] have shown that cold housing of rats causes remarkable changes in substrate specificity of brain MAO-A: it becomes capable of desaminating unusual substrates such as glucosamine. Prelimi-

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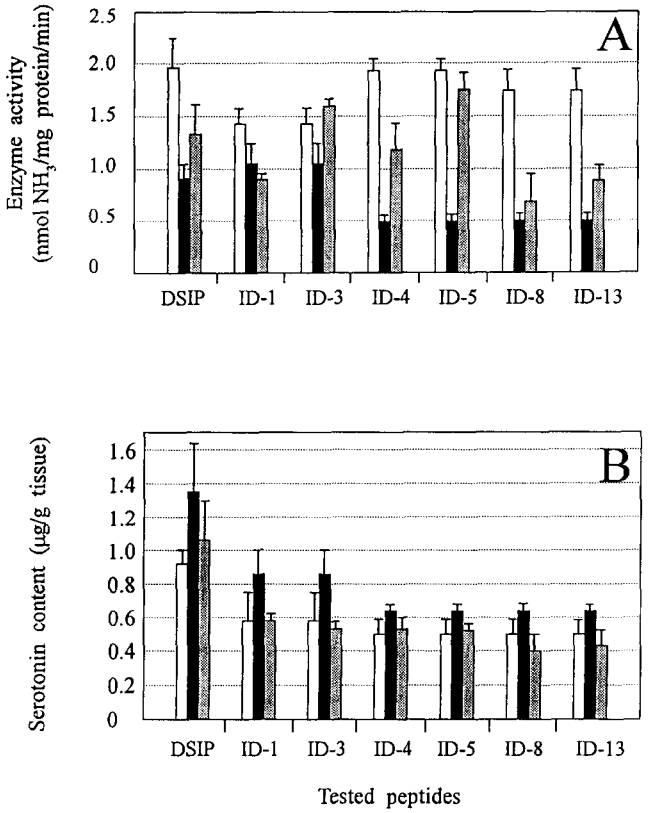

Fig. 1. (A) Activity of MAO-A in mitochondrial fractions from brain of the following groups of rats: (1) intact (open bars); (2) subjected to hypoxia (filled bars); (3) pretreated with tested peptides (120 μg/kg, i.p.) before hypoxia stress (hatched bars). (B) Concentration of serotonin in brain of rats from the same groups. Experiments for analogues were done separately throughout a period of several months and this may be a reason of remarkable deviations in initial MAO-A activity and serotonin content in brain of rats under hypoxia. Results are expressed as means ± S.E.M. for each group of animals (n = 6–8).

nary injection of DSIP (i.p., 120 μg/kg) into rats subjected to cold mitigated stress-induced changes in activity of this enzyme.

Therefore in present study we assessed the influence of DSIP and its analogues on the activity of mitochondrial MAO type A and the level of its substrate serotonin in rat brain under experimental hypoxia. In accordance with the data, presented in the Table MAO-A exhibited the activity only in mitochondrial fraction and this activity was practically not found in cytoplasmic fraction obtained from the brain of intact animals. Injection of DSIP to intact rats did not significantly influence MAO-A activity in the brain. Subjection of rats to hypoxia conditions leads to decrease of MAO-A activity in mitochondria and its adventing in cytoplasme. Preliminary injection of DSIP (i.p., 120 μg/kg) to rats before stressful shortterm housing in reduced atmosphere partially inhibited hypoxia induced changes. Mitochondrial MAO-A activity was increased about 46% in comparison with DSIP-untreated rats under hypoxia although it did not reach the values corresponding to intact animals. At the same time the opposite effect of DSIP on MAO-A activity in cytoplasme may be traced. In addition DSIP has a tendency to normalize the serotonin content in brain of rats under hypoxia (Table 1). All these effects caused by DSIP under hypoxia are not fully significant but they may be considered as a definite tendency of this peptide to mitigate stress-induced changes in MAO-A activity.

It seems reasonable to say that decrease of MAO-A activity in mitochondria may be connected with activation of lipid peroxidation as a key event caused by stressful stimuli. Changing lipid environment may influence the MAO-A activity. The first evidence implies that association of the enzyme with outer mitochondrial membrane is disrupted. This event is followed by MAO-A release to the cytoplasme. Preventive effect of DSIP might be attributed to ability of this peptide to decrease the intensity of lipid membrane peroxidation [20] and thereby to retain the association of enzyme with mitochondrial membranes.

Thus, we may conclude that DSIP partially restricted hypoxia-induced changes in MAO-A activity of brain and these effects are in a good agreement with the ability of this peptide to retard undesirable metabolic deviations under stressful conditions mentioned above.

In order to shed more light to relationships between structure and biological activity we investigated the related effects of DSIP analogues in comparison with aforementioned action of DSIP in rats under hypoxia. Therefore a number of DSIP analogues varying in positions 1, 2 and 6 were tested:

	1	2	3	4	5	6	7	8	9
DSIP	Trp-	Ala-	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu
ID-1	Trp-	**DVal**	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu
ID-3	Trp-	**DAla**	-Gly-	-Gly-	-Asp-	**Tyr**	-Ser-	-Gly-	-Glu
ID-4	Trp-	**DLeu**	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu
ID-5	**Tyr-**	**Pro**	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu
ID-8	**Tyr-**	**DAla**	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu
ID-13	Trp-	**DPhe**	-Gly-	-Gly-	-Asp-	-Ala-	-Ser-	-Gly-	-Glu

To compare efficiency of these analogues we analyzed the changes in activity of mitochondrial MAO-A and also in the content of serotonin in rat brain after their injection before hypoxia manipulation (Fig. 1A,B). The data obtained we compared with corresponding values for rats subjected to hypoxia without preliminary injection of analogues. We found analogues ID-3 and ID-5 much more active than DSIP. They almost fully prevented stress-induced changes in mitochondrial

Table 1
Influence of DSIP pretreatment (120 μg/kg, i.p.) on serotonin content and distribution of MAO-A activity between mitochondrial and cytoplasmic fractions from rat brain under hypoxia stress

Group of animals	Activity of MAO-A (nmol NH_3/mg protein/min)		The content of serotonin in rat brain (μg/kg tissue)
	Mitochondrial fraction	Cytoplasmic fraction	
Intact	1.96 ± 0.28 (n = 5)	nd	0.92 ± 0.08 (n = 13)
Pretreated with DSIP	2.48 ± 0.26 (n = 4)	nd	1.00 ± 0.04 (n = 6)
Subjected to hypoxia	0.91 ± 0.13 (n = 6)	1.15 ± 0.37 (n = 6)	1.35 ± 0.30 (n = 6)
Subjected to hypoxia and pretreated with DSIP	1.33 ± 0.28 (n = 7)	0.70 ± 0.32 (n = 7)	1.06 ± 0.25 (n = 6)

Values represent mean ± S.E.M. The number of animals is given in parentheses; nd, not detected.

MAO-A activity and serotonin content in rat brain. Similar action on serotonin level was found for all other tested analogues. Analogues ID-1 and ID-8 were inactive in relation to MAO-A activity.

It follows from the above results that activity of DSIP is sensitive to structural alterations of the molecule. Replacement of Trp by Tyr in position 1 of the DSIP molecule together with introduction of Pro residue in position 2 (analogue ID-5), considerably increases the efficiency. Replacement of Ala by Tyr in position 6 together with introduction of D-Ala residue in position 2 (analogue ID-3) also increases the activity. Rather unexpectedly, replacement of Trp by Tyr in position 1 of the DSIP molecule together with introduction of D-Ala residue in position 2 (analogue ID-8), led to drastic loss of the efficiency. Increase of hydrophobicity in position 2 (replacement of Ala by DVal, DPhe or DLeu) had no effect on activity (analogues ID-4 and ID-13), or resulted in loss of the efficiency (analogue ID-1) in comparison with DSIP. It should be noted that the analogue ID-5 was found to be active also as antimetastatic and antiepileptic agent [9,12]. The changes in activity of tested analogues might be considered as a consequence of their conformational pecularities influencing probably membrane-protective action of this amphiphilic endogenous peptide.

Thus we may conclude that DSIP and its analogues can modulate changes in brain mitochondrial MAO-A activity induced by experimental hypoxia in rats. Established effects are presumably associated with membrane-protective properties of DSIP and some its analogues. These findings may contribute to studies on undirect DSIP actions under stress. The problem of how this peptide facilitates resistance of animals to stress conditions requires further investigation.

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