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The Role of Neurotransmitters in Protecting Brain during Acute Hypoxia

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(Presented by Academy Member David Mikeladze)

The work is devoted to the study of the influence of the precursors of dopamine, norepinephrine (L-DOPA), serotonin (5-hydroxytryptophan) and GABA (sodium oxybutyrate) on the accumulation of lipid peroxidation products (LPO), the activity of ATPase enzymes and the content of various types of SH-groups in the visual cortex and medulla oblongata during acute hypoxia. It was found that acute hypoxia leads to an increase in LPO products (hydroperoxides and malonic aldehyde), a decrease in the activity of transport ATPases and the content of various types of SH-groups. Preliminary administration of various doses of L-DOPA (20, 40 and 80 mg/kg live weight), 5-hydroxytryptophan (10 mg/kg live weight) and sodium oxybutyrate (100 mg/kg live weight) to animals during hypoxia and hypoxia with reoxygenation almost completely suppressed the decrease of ATPase enzymes activity and the content of various types of SH-groups in the visual cortex and medulla oblongata. It is assumed that the physicochemical mechanism of the protective action of inhibitory neurotransmitters under the action of stress factors is based on their antioxidant function.

Neurotransmitters, SH-groups, lipid peroxidation products, Na^+, K^+ - ATPase

The modern approach to establishing the neurochemical criteria for resistance to stress factors is characterized by a significant concentration of efforts in the field of creating bioregulators based on peptides with antioxidant and adaptogenic properties, studying their effectiveness and justifying the appropriateness in the prevention and treatment of various pathological conditions, including when studying the effects of hypoxia/brain ischemia [1, 2]. It is known, that the adaptive and protective ability of the body to stress factors includes the mechanisms of action at the systemic, cellular and molecular level. An important role in this complex defense system is played by mediators (GABA (γ-aminobutyric acid), dopamine, serotonin, opioid peptides etc. [3-5]), the physicochemical mechanisms of action of which are not completely identified yet. Considering that one of the main damaging factors in stress is the intensification of lipid peroxidation (LP) [5, 6], it can be assumed that the protective mechanism of mediators under the influence of extreme factors on the body is carried

out by stabilizing free radical reactions. The role of neuropeptides as antihypoxants is well known: a change in the balance of activating and inhibitory mediators entails a change in the state of peroxidation processes in the structures of the brain [7]. Mediators and their in vitro precursors effectively suppress LP intensity in various membrane formations [8]. Also, for the normal functioning of the brain, timely detoxification of LPO products is required, which occurs with the direct participation of thiol compounds (SH-groups). The antioxidant role of glutathione (GSH) in this process is very important, since GSH makes up the majority of low molecular weight thiols in brain cells [9]. As a sensitive biomarker of oxidative stress, it is possible to use a change in the ratio of the sum of all low molecular weight thiols bound to plasma proteins to protein-free cysteinyl residues [10]. Based on the foregoing, in the present work, the effect of the precursors of dopamine, serotonin, and GABA (γ-aminobutyric acid) – L-DOPA, 5-hydroxytryptophan and sodium oxybutyrate (GHB sodium salt), respectively, on the accumulation of lipid peroxidation products is investigated, as well as the typical for intensification LPO suppression of the activity of transport ATPases and changes in the content of various types of SH-groups in the visual region of the cortex and medulla oblongata.

Materials and Methods

The experiments were conducted on 84 white outbred rats (200 ± 25 g). The animals were kept in a vivarium under natural lighting conditions with free access to water and food. The study was performed according to the principles expressed in the Declaration of Helsinki revised by WMA, Fortaleza, Brazil, 2013. Considering that neurotransmitters do not cross the blood-brain barrier (BBB), in order to increase dopamine, norepinephrine, serotonin and GABA in the brain, animals (except control group) were administered L-DOPA at a dose of 20, 40 and 80 mg/kg live weight an hour before hypoxic exposure, 5-hydroxytryptophan (5-HTP) at a dose of 10 mg/kg live weight and sodium oxybutyrate (sodium salt of γ-hydroxybutyric acid) at a dose of 100 mg/kg live weight. The first two substances were administered intraperitoneally, and the last – orally. All doses of L-DOPA introduced cause almost the same changes. Therefore, the Tables show only the results of those injected by 40 mg/kg body weight. Teturam (Disulfiram) in the form of an emulsion in starch was administered intraperitoneally at a dose of 100 mg/kg body weight for 2.5 hours before the injection of L-Dopa. Acute hypoxia was created in a hermetically sealed chamber with a volume of 0.12 m^3 for 1.5 hours by constantly pumping a pre-prepared gas mixture (5% O_2 and 95% N_2) through it. Reoxygenation was carried out by purging the chamber with oxygen under slight pressure for 20 min. Immediately after hypoxia and hypoxia with reoxygenation, animals were decapitated, and the indicated brain regions were removed. The content of LPO products, namely hydroperoxides (HP) and malondialdehyde (MDA), was determined by the Asakawa method [11]. The change in the content of GP and MDA was expressed in relative units and nmol/mg protein, respectively. Determination of the activity of Na, K- and Mg-ATPases was carried out as in the work of Bonting et al [12] and was expressed in μmol Pi/mg protein/45 min. Determination of total (using 1% detergent sodium dodecyl sulfate), protein-bound and nonprotein- glutathione (GSH) SH-groups was performed according to the method of Ellman described in the work of Sedlak and Lindsay [13]. The content of SH- groups was expressed in μmol/mg protein. Protein concentration was measured by the Lowry method in the modification of Miller [14]. All results are presented as means ± SEM (M ± m). The significance of the results was determined by a Student t-test and evaluation of the significance of differences was carried out at $p < 0.05$. The analyses were carried out using the statistical software package MS Excel.

Results and Discussion

It has been shown that the action of acute hypoxia leads to an increase in the content of LPO products, which is accompanied by a decrease in the activity of transport ATPases and the content of various types of SH- groups in the visual cortex and medulla oblongata (Tables 1, 2, 3). Moreover, in the medulla oblongata, the content of HP was 1.4 times higher, and the accumulation of MDA was insignificantly lower than in the visual cortex. Reoxygenation after acute hypoxia led to a significant increase in lipid peroxidation products, which proceeded more intensively in the tissues of the medulla oblongata. Preliminary administration of L-DOPA and sodium oxybutyrate to animals during hypoxia and hypoxia with reoxygenation completely suppressed the accumulation of lipid peroxidation products (Table 1). To differentiate the effect of dopamine and norepinephrine under the action of L-DOPA, an inhibitor of the conversion of dopamine to norepinephrine teturam was used. It was found that the antioxidant capacity of L-DOPA is reduced by approximately 65%. Therefore, it can be assumed that the antioxidant effect of L-DOPA under the action of hypoxia is carried out by both dopamine and norepinephrine.

Under the action of hypoxia and hypoxia with reoxygenation, decrease in the content of all fractions of SH– groups and suppression of the activity of transport ATPases in the medulla oblongata and in the visual cortex were found (Tables 2, 3). Preliminary administration of neurotransmitters significantly inhibited the action of hypoxia and hypoxia with reoxygenation. Despite this, against the background of an increase in the content of total thiol fractions, there was a slight decrease in the level of glutathione and the activity of Na^+, K^+ - ATPase in comparison with the control. SH- groups occupy a special place in the regulation of lipid peroxidation, being the first line of defense against oxidative stress. In particular, glutathione in the presence of the enzyme glutathione peroxidase is involved in the detoxification of hydrogen peroxide and lipid hydroperoxide, and thus exhibits antioxidant properties. In addition, the high sensitivity of SH-groups to the action of LPO products is the reason for the suppression of the activity of many enzymes. It is known that the addition of

Table 1. Changes in the content of HP and MDA in the medulla oblongata and visual cortex during acute hypoxia. ((M±m) n= 7-9)

№	Experiment Conditions	Medulla oblongata		Visual cortex	
		HP (relative unit)	MDA (nmol/mg protein)	HP (relative unit)	MDA (nmol/mg protein)
1	Control group	3,85 ± 0,21	3,00 ± 0,12	4,12 ± 0,40	2,72 ± 0,17
2	Hypoxia p 2-1	7,17 ± 0,20 < 0,05	6,10 ± 0,60 < 0,05	5,30 ± 0,34 < 0,01	6,4 1± 0,15 < 0,05
3	Hypoxia + reoxygenation p 3-2	7,57 ± 0,23 < 0,05	6,97 ± 0,75 < 0,05	6,34 ± 0,60 < 0,05	6,80 ± 0,51< 0,05
4	L-DOPA + hypoxia p 4-2	3,90 ± 0,11 < 0,05	2,16 ± 0,32 < 0,05	4,13 ± 0,13 < 0,05	1,98 ± 0,11 < 0,05
5	L-DOPA + hypoxia + reoxygenation p 5-3	4,20 ± 0,24 <0,01	2,68 ± 0,31 < 0,05	4,47 ± 0,10 < 0,05	2,39 ± 0,09 < 0,01
6	5-hydroxytryptophan + hypoxia p 6-2	4,77± 0,13 < 0,05	3,62 ± 0,12 < 0,05	4,88 ± 0,14 < 0,05	3,91 ± 0,18 < 0,05
7	5-hydroxytryptophan + hypoxia + reoxygenation p 7-3	5,13 ± 0,47 < 0,05	4,55 ± 0,22 < 0,05	5,21 ± 0,22 < 0,01	3,93 ± 0,24 < 0,05
8	Sodium oxybutyrate + hypoxia p 8-2	4,37± 0,22 < 0,05	2,94 ± 0,65 < 0,01	4,46 ± 0,13 < 0,01	2,61 ± 0,11 < 0,01
9	Sodium oxybutyrate + hypoxia + reoxygenation p 9-3	4,51 ± 0,32 < 0,01	3,05 ± 0,12 < 0,01	4,79 ± 0,36 < 0,05	2,48 ± 0,24 < 0,05

Table 2. Changes in the activity of transport ATPases in the medulla oblongata and optic cortex in acute hypoxia ((M±m) n= 7-9)

№	Experiment Conditions	Medulla oblongata		Visual cortex	
		ATPase activity in µmol Pi / mg protein / 45 min			
		Na^+, K^+ ATPases	Mg^{+2} ATPases	Na^+, K^+ ATPases	Mg^{+2} ATPase
1	Control group	8,12 ± 0,29	17,24 ± 1,62	7,67 ± 0,92	19,12 ± 1,44
2	Hypoxia p 2-1	6,46 ± 1,31 < 0,05	14,67 ± 1,10 < 0,01	6,86 ± 0,41< 0,05	17,78 ± 0,92 < 0,05
3	Hypoxia + reoxygenation p 3-2	5,87 ± 0,12 < 0,005	13,65 ± 1,14 < 0,05	5,69 ± 0,22 < 0,05	15,34 ± 1,27 < 0,05
4	L-DOPA + hypoxia p 4-2	7,35 ± 0,27 < 0,05	19,32 ± 1,26 < 0,05	6,79 ± 0,51< 0,05	20,29 ± 1,25 < 0,05
5	L-DOPA + hypoxia+ reoxygenation P 5-3	8,23 ± 0,13 < 0,05	17,34 ± 1,26 < 0,05	8,21 ± 0,44 < 0,05	18,25 ± 0,75 < 0,05
6	5-hydroxytryptophan +hypoxia p 6-2	7,47 ± 0,42 < 0,05	17,12 ± 1,53 < 0,05	7,26 ± 0,22 < 0,01	18,23 ± 0,79 < 0,05
7	5-hydroxytryptophan +hypoxia+ reoxygenation p 7-3	8,21 ± 0,71 < 0,05	17,52 ± 1,67 < 0,05	7,41 ± 0,13 < 0,05	18,17 ± 0,15 < 0,01
8	Sodium oxybutyrate + hypoxia p 8-2	7,24 ± 0,91 < 0,05	17,26 ± 0,13 < 0,01	6,79 ± 0,52 < 0,05	18,19 ±1.22 < 0,01
9	Sodium oxybutyrate + hypoxia+ reoxygenation p 9-3	7,58 ± 0,39 < 0,05	17,47 ± 1,16 < 0,05	7,31 ± 0,84 < 0,01	17,14 ±1,30 < 0,05

Table 3. Changes in the content various SH-groups in the medulla oblongata and optic cortex in acute hypoxia ((M±m) n= 7-9)

№	Experiment Conditions	Medulla oblongata			Visual cortex		
		SH-groups µmol/mg protein			SH-groups µmol/mg protein		
		Total	Protein-bond	GSH	Total	Protein-bond	GSH
1	Control group	62,13 ± 5,16	28,23 ± 2,10	15,97 ± 3,66	57,97 ± 3,31	25,84 ± 2,13	15,83 ± 1,23
2	Hypoxia p 2-1	45,21 ± 4,10 < 0,05	23,73 ± 1,76 < 0,05	12,46 ± 1,30 < 0,05	39,82 ± 3,22 < 0,05	20,88 ± 1,15 < 0,05	13,79 ± 1,13 < 0,05
3	Hypoxia + reoxygenation p 3-2	45,28 ± 3,22 < 0,05	21,83 ± 2,68 < 0,05	11,92 ± 1,30 < 0,05	38,58 ± 2,18 < 0,05	18,95 ± 1,12 < 0,01	9,92 ± 0,68 < 0,01
4	L-DOPA + hypoxia p 4-2	63,92 ± 4,77 < 0,05	27,96 ± 2,31 < 0,01	14,98 ± 1,17 < 0,05	57,83 ± 4,16 < 0,05	24,77 ± 1,63 < 0,01	15,47 ± 1,30 < 0,05
5	L-DOPA + hypoxia + reoxygenation P 5-3	64,67 ± 4,13 < 0,05	28,57 ± 3,44 < 0,05	15,89 ± 1,10 < 0.05	50,89 ± 3,18 < 0,05	24,76 ± 2,11 < 0,05	13,89 ± 1,14 < 0,05
6	5-hydroxytryptophan + hypoxia p 6-2	61,12 ± 2,31 < 0,05	27,66 ± 0,24 < 0,01	13,87 ± 1,14 < 0,05	55,73 ± 3,80 < 0,05	22,96 ± 1,19 < 0,05	13,97 ± 1,32 < 0,05
7	5-hydroxytryptophan + hypoxia + reoxygenation p7-3	61.96 ± 3,01 < 0,05	27,81 ± 0,21 < 0,05	14,93 ± 1,16 < 0,05	57,95 ± 3,13 < 0,01	25,51 ± 2,33 < 0,05	15,67 ± 1,27 < 0,01
8	Sodium oxybutyrate + hypoxia p8-2	58,66 ± 2,11 < 0,05	25,94 ± 0,21 < 0,05	14.73 ± 1,31 < 0,01	54,77 ± 2,48 < 0,05	25,84 ± 2,10 < 0,01	14,64 ± 1,33 < 0,01
9	Sodium oxybutyrate + hypoxia + reoxygenation p9-3	63,32 ± 3,00 < 0,05	24,95 ± 0,20 < 0,05	14,81 ± 1,32 < 0,05	56,96 ± 4,14 < 0,05	26,73 ± 0,38 < 0,05	14,69 ± 1,34 < 0,05

glutathione to the cysteine of the α-subunit of the atphase catalytic center leads to inhibition of the enzyme activity and ROS generation. In addition, the high sensitivity of SH- groups to the action of LPO products is the reason for the suppression of the activity of many enzymes [15].

Conclusion

Considering the above, as well as the fact that it was in the brain tissue that the most stable to oxidation of the α-subunit of the enzyme isoform was found (one SH- group less than in isoforms of enzymes found in other tissues), in this case, a decrease in

activity of Na^+, K^+ - ATPases can be explained by an increase in the content of sulfhydryl groups of glutathione due to the antioxidant effect of neurotransmitters. Thus, it can be assumed that the physicochemical mechanism of the protective action of mediators during hypoxia is based on their antioxidant function, which is expressed both in the effective suppression of the accumulation of lipid peroxidation products, and in the prevention of suppression of the activity of transport ATPases and a decrease in the content of various SH– groups in brain tissues.

ბიოფიზიკა

ნეიროტრანსმიტერების როლი ტვინის დაცვაში მწვავე ჰიპოქსიის დროს

ს. ჯაფაროვა

აზერბაიჯანის მეცნიერებათა ეროვნული აკადემია, ბიოფიზიკის ინსტიტუტი, ეკოლოგიური ბიოფიზიკის ლაბორატორია, ბაქო, აზერბაიჯანი

(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

შესწავლილია დოფამინის, ნორეპინეფრინის (L-დოფა), სეროტონინის (5-ჰიდროქსიტრიფტოფანი) და გამკის (ნატრიუმის ოქსიბუტირატი) გავლენა ლიპიდების (პოლ) ზეჟანგური დაჟანგვის პროდუქტების ეფექტზე, ATP-აზების აქტივობაზე, ასევე თავის ტვინის ქერქის მხედველობითი უბნისა და მოგრძო ტვინის SH-ჯგუფებზე მწვავე ჰიპოქსიის პირობებში. დადგენილია, რომ მწვავე ჰიპოქსია ზრდის პოლ-ის პროდუქტებს (ჰიდროზეჟანგები და მალონის დიალდეჰიდი), ამცირებს ტრანსპორტული ATP-აზების აქტივობას და სხვადასხვა ტიპის SH-ჯგუფების დონეს. ცხოველებში L-დოფას სხვადასხვა დოზის (20,40 და 80 მგ/კგ ცოცხალ წონაზე) წინასწარი შეყვანა, ასევე 5-ჰიდროქსიტრიფტოფანის (10 მგ/კგ ცოცხალ წონაზე) და ნატრიუმის ოქსიბუტირატის (100 მგ/კგ ცოცხალ წონაზე) ჰიპოქსიისა და ჰიპოქსიის რეოქსიგენაციით, პრაქტიკულად მთლიანად აფერხებს პოლ-ის პროდუქტების დაგროვებას, რაც გარკვეულწილად იცავს ATP-აზების აქტივობასა და SH-ჯგუფების სტაბილურობას ქერქის მხედველობის ზონასა და მოგრძო ტვინში. სავარაუდოა, რომ სტრესოგენური ფაქტორების ზემოქმედებისგან აღნიშნული ნეირომედიატორების დაცვით მექანიზმს საფუძვლად უდევს მათი ანტიოქსიდანტური ფუნქცია.

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