EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

In Vitro Response of Mitochondrial Succinate Oxidase System to Epinephrine in Human Blood Lymphocytes from Health Individuals and Patients with Neurocirculatory Dystonia

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Activities of succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (hyaloplasmic and mitochondrial: α-$GPDH_H$ and α-$GPDH_M$) in peripheral blood lymphocytes, and the response of SDH activity to exogenous epinephrine *in vitro* (the epinephrine test) were studied in 20 healthy subjects and 46 patients with hypertensive neurocirculatory dystonia. Activities of SDH, α-$GPDH_H$, and α-$GPDH_M$ in blood lymphocytes and SDH adrenoreactivity in epinephrine test were higher in patients than in healthy controls. Treatment with hypotensive agents (isradipin and pyrroxan) moderated adrenoreactivity. Phytotherapy normalized the baseline activities of succinate oxidase system and α-glycerophosphate pathway in blood lymphocytes.

Key Words: *epinephrine; succinate dehydrogenase; α-glycerophosphate dehydrogenase; neurocirculatory dystonia; adrenoreactivity*

The level of activation of mitochondrial succinate oxidase system is a very sensitive parameter characterizing the state of cell respiration during hypoxia [1,5].

Epinephrine increases activity of succinate dehydrogenase (SDH) both *in vitro* [8] and *in vivo* [8,9]. This effect can be explained by enhanced succinic acid oxidation [2]. Norepinephrine and indraline (α_{1B}-adrenomimetic) produce similar effects [4,11]. Pharmacological effect of epinephrine and norepinephrine on SDH activity is opposite to that of acetylcholine [7, 11]. Epinephrine-induced activation of SDH is associated with activation of the adenylate cyclase system (cAMP/cGMP ratio increases) [3]. α-Adrenoblocker tropaphen abolishes the reaction of lymphocyte SDH to α_{1B}-adrenomimetic indraline [4,8].

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The temporal and dose-dependent characteristics of the reaction of lymphocyte succinate oxidase system to epinephrine, α_{1B}-adrenomimetic indraline, and hypoxic hypoxia were studied on mice, rats, dogs, and human volunteers [1,4,9]. Our aim was to study adrenoreactivity of succinate oxidase system in healthy subjects and patients with neurocirculatory dystonia (NCD).

MATERIALS AND METHODS

The study was performed on healthy donors (n=20) and male patients with NDC aged 25-40 years (n=54, mean 34±1 year). Venous blood was taken after overnight fast, and blood pressure (BP) was measured.

Adrenoreactivity of the organism was assessed by epinephrine test [8]. Venous blood (1 ml) in heparini-

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zed tube (25 U) was kept at 37°C. Epinephrine (0.01%, 10 μl) was added to blood (final concentration 1 μg/ml). Blood smears for measuring SDH activity were prepared before and 2 min after addition of epinephrine. Adrenoreactivity of the organism was evaluated by the increment of SDH activity in response to epinephrine *in vitro*:

$$\frac{(SDH_{epineph}\text{-}SDH_{baseline})}{SDH_{baseline}} \times 100\%.$$

Activities of SDH and α-glycerophosphate dehydrogenase (hyaloplasmic and mitochondrial: α-$GPDH_H$ and α-$GPDH_M$, respectively) in blood lymphocytes were evaluated histochemically by the mean number of diformazan granules per cell formed in the redox reaction with p-nitroviolet tetrazolium [12]. Diformazan granules were counted in no less that 50 lymphocytes in two blood smears.

Cytochemical study was carried out before and after treatment of NCD patients with hypotensive drugs isradipin (1.25 mg daily) and pyrroxan (0.015 mg daily) for 10-12 days or phytotherapy for 3-4 weeks. Herbal tincture was prepared from medical herbs: calendula flowers (1.5 g), common origanum (2 g), stinging nettle (2 g), dog-rose fruits (2 g) per 200 ml water; alcohol extracts of leuzea (3 ml) and haw (3 ml) were added. The tincture (100 ml) was administered 2 times a day: 1 h before and 2 h after meal.

The results were analyzed statistically using Student's *t* test.

RESULTS

The concentration dependence of SDH reaction in blood lymphocyte to epinephrine *in vitro* is logarithmic and can be approximated by the following equation:

$$SDH_{epineph}=1.74\times\ln(x)+32.34 \ (r=0.59, \ p<0.001),$$

where x is epinephrine concentration in the incubation medium.

In comparison with healthy controls, NCD patients had higher BP, higher baseline activities of SDH, α-$GPDH_H$, and α-$GPDH_M$ in blood lymphocytes, and more pronounced reaction of lymphocyte SDH to epinephrine (Table 1).

The initial level of SDH in blood lymphocytes most closely correlated with BP and α-GPDH activity (Table 2). Multiple correlation analysis describes this dependence by a polynomial formula:

$$SDH=8.82\text{-}0.07\alpha\text{-}GPDH_M+0.27\ \alpha\text{-}GPDH_H+0.08SDH_{epineph}+0.07DAP+0.07SAP,$$
$$(r=0.70, \ p<0.01).$$

The positive correlation between initial SDH and α-$GPDH_H$ activities and BP is significant. It is described by the following formulas:

$$SDH=2.58+0.16DAP+0.13SAP$$
$$(r=0.45, \ p<0.05),$$
$$\alpha\text{-}GPDH_H=0.37DAP+0.22SAP-31.9$$
$$(r=0.42, \ p<0.05).$$

There is a positive correlation between ($SDH_{epineph}$) and initial activity of lymphocyte SDH and α-GPDH:

$$SDH_{epineph}=16.4+0.31SDH+\ 0.61\alpha\text{-}GPDH_M+0.03\alpha\text{-}GPDH_H,$$
$$(r=0.66, \ p<0.01).$$

In NCD patients the epinephrine-induced increment of SDH activity before treatment was more pronounced compared to healthy volunteers (Table 3, $p<0.01$). Treatment with hypotensive drugs normalized BP and decreased adrenoreactivity of lymphocyte SDH to normal, but baseline SDH activity remained high against the background of increased activity of the α-glycerophosphate pathway (by about 1.4-fold

TABLE 1. Initial Activities of SDH and α-GPDH in Blood Lymphocytes from Healthy Volunteers and NCD Patients (*M*±*m*)

Index		Healthy volunteers	NCD patients
SDH activity, arb. units	baseline	28.9±0.4	31.0±0.5*
	$SDH_{epineph}$	35.0±0.8+	38.3±0.9*+
Adrenoreactivity, %		19.7±2.6	23.6±2.7
α-$GPDH_H$ activity, arb. units		19.2±0.6	24.3±0.9*
α-$GPDH_M$ activity, arb. units		15.5±0.7	17.8±0.8*
DAP, mm Hg		76.3±1.1	82.9±1.2*
SAP, mm Hg		118.8±1.7	128.3±1.5*

Note. $p<0.05$: compared to *healthy controls and +baseline value.

TABLE 2. Correlation between Physiological and Biochemical Indices of Healthy Volunteers and NCD Patients

Index	DAP	SAP	α-GPDH$_H$	SDH$_{epineph}$	SDH$_{epineph}$
SDH	0.31*	0.34*	0.66*	0.55*	0.47*
α-GPDH$_H$	0.32*	0.27	1	0.85*	0.59*
α-GPDH$_M$	0.25	0.27	0.85*	1	0.65*
SDH$_{epineph}$	0.12	0.18	0.47*	0.59*	1

Note. *Correlation is significant (p<0.05).

judging from α-GPDH activity, Table 3). Thus, the epinephrine test assesses cell adrenoreactivity in NCD patients during therapy by *in vitro* reaction of SDH in blood lymphocytes to epinephrine.

Phytotherapy decreased SAD and DAP and normalized the baseline activity of SDH and α-GPDH (Table 3). Thus, combined therapy with plant preparations producing sedative, vasodilator, hypoaggregant, and antiatherogenic effects is pathogenetically adequate for the treatment of NCD patients.

There is evidence on enhanced initial level of SDH and α-GPDH in peripheral blood lymphocytes in NCD patients [6]. Activation of SDH and α-glycerophosphate pathway is an adequate adaptive response in some pathophysiological states, *e.g.* NCD, and is associated with adrenergic hyperstimulation.

It is hypothesized that the development of hypertensive states during NCD and essential hypertension results from enhanced sensitivity of the organism (in particular, its vascular system) to catecholamines. Hypertensive animals demonstrate enhanced reacti-vity to exogenous epinephrine [13], while repeated injections of norepinephrine to humans potentiate the metabolic response to this catecholamine [15]. The proposed epinephrine test based on activation of SDH by exogenous epinephrine reveals enhanced reactivity to catecholamines in patients with hypertension and NCD.

Activation of this metabolic pathway during pathophysiological states closely correlates with intensification of tissue respiration and predominant use of lipids as the source of oxidation [2,10,14]. Under these conditions, lipid metabolism is aimed at the synthesis of glucose and glycogen and obligatorily involves activation of succinate oxidase system in cells [10]. Most likely, this chain of events realizes the trophic function of the adrenergic system manifesting in activation of glucose synthesis from glycogen and lipids, and glycolytic and oxidative generation of ATP, which is required for adaptive rearrangements in the organism in response to stress stimulation.

Stress and administration of epinephrine or adrenomimetics induce typical reactions in humans and animals manifested in repeated rise of SDH activity [1,3,5,9]. A high potency of this mechanism is underlain by buffer capacity of the processes related to synthesis of succinic acid (oxidation substrate), which supports cell respiration by intensification of oxidative phosphorylation and "soft" disintegration coupled with up-regulation of free oxidation at the first stage of metabolic hypoxia.

Pharmacotherapy of NCD with hypotensive drugs moderates adrenoreactivity of the organism, albeit it does not normalize the metabolic processes in cells. Treatment with calcium channel blockers notably enhances the load to α-glycerophosphate pathway, which

TABLE 3. Medical Effects of Hypotensive Drugs and Phytotherapy on BP and SDH and α-GPDH Activities in Blood Lymphocyte from NCD Patients ($M\pm m$)

Index		Pharmacotherapy (n=16)		Phytotherapy (n=12)	
		before treatment	after treatment	before treatment	after treatment
SDH activity, rel. units	baseline	32.1±1.0	31.8±1.3	29.4±0.3	26.5±0.4*
	SDH$_{epineph}$	43.6±1.6$^+$	38.3±1.2*$^+$	—	—
Adrenoreactivity, %		39.7±4.6	18.7±4.7*	—	—
α-GPDH$_H$ activity, rel. units		33.1±1.9	46.7±4.8*	23.5±0.5	*21.7±0.4
α-GPDH$_M$ activity, rel. units		26.5±1.4	36.5±4.7	16.2±0.5	*14.6±0.5
DAP, mm Hg		88.1±2.1	78.8±1.6*	87.1±1.0	80.0±1.4*
SAP, mm Hg		139.4±4.1	126.9±1.6*	136.2±2.5	128.1±1.3*

Note. p<0.05: compared to *indices before treatment and $^+$baseline values.

transfers reduced equivalents from cytosol into mitochondria, which can be related to activation of glycolysis or pentose-phosphate cycle. By contrast, in addition to BP decrease, phytotherapy normalizes baseline activity of succinate oxidase system and the catecholamine of α-glycerophosphate pathway in cells.

REFERENCES

1. V. V. Antipov, M. V. Vasin, and A. N. Gaidamakin, *Kosm. Biol. Aviakosm. Med.*, **23**, No. 2, 63-66 (1989).
2. A. M. Babskii, M. N. Kondrashova, and I. V. Shostakovskaya, *Fiziol. Zh.*, **31**, No. 3, 301-306 (1985).
3. M. V. Vasin, T. V, Petrova, and L. V. Koroleva, *Ibid.*, **77**, No. 4, 106-108 (1991).
4. M. V. Vasin, I. B. Ushakov, L. V. Koroleva, and V. V. Antipov, *Radiobiologiya*, **39**, Nos. 2-3, 238-248 (1999).
5. A. N. Gaidamakin and M. M. Abramov, *Ibid.*, **27**, No. 4, 524-528 (1987).
6. S. L. Denisov, L. V. Koroleva, and I. A. Lairov, *Aviakosm. Ekol. Med.*, **30**, No. 1, 44-46 (1996).
7. M. N. Kondrashova, *Biokhimiya*, **56**, 388-405 (1991).
8. L. V. Koroleva, *Radiation Sensitivity of Biologic Organism and Radioprotection Efficiency under the Combined Effects of Ionizing Radiation and Normobaric Hyperoxia*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1990).
9. L. V. Koroleva and M. V. Vasin, *Radiobiologiya*, **28**, No. 2, 228-230 (1988).
10. N. P. Lebkova, *Vestn. Ross. Akad. Med. Nauk*, No. 9, 16-22 (2000).
11. L. A. Lyubovtseva, in: *Tissue Morphology and Histochemistry in Norm, Pathology, and Experiment* [in Russian], Cheboksary (1982), pp. 10-12.
12. A. V. Merkur'eva, G. L. Bilich, and R. P. Nartsissov, *Methodical Hints (Biochemical and Cytochemical Methods to Determine Activity of Enzymes and Enzyme-Substrate Systems in Various Cell Compartments)* [in Russian], Vol. 1, Moscow, Ioshkar-Ola (1982).
13. G. S. Yakobson, A. R. Antonov, G. V. Petrova, *et al., Byull. Eksp. Biol. Med.,* **121**, No. 5, 495-498 (1996).
14. G. Faintrenie and A. Geloen, *J. Pharmacol. Exp. Ther.,* **286**, No. 2, 607-610 (1998).
15. A. V. Kurpad and R. N. Kulkarni, *Metabolism,* **38**, No. 10, 979-982 (1989).