BIOPHYSICS AND BIOCHEMISTRY

Effect of Intraperitoneal Spermin on Oxidative Processes in Isolated Mitochondria of Rat Liver in Hypothermia

N. A. Lukoyanova and I. S. Meilanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 124, No. 5, pp. 526-528, May, 1998
Original article submitted July 15, 1997

Effect of intraperitoneal administration of spermin on oxidative phosphorylation and calcium capacity of isolated liver mitochondria was studied in normo- and hypothermic rats. Hypothermia stimulates mitochondrial respiration without decreasing the contingency and increases calcium capacity. Spermin suppresses mitochondrial respiration, the effect being stronger in hypothermia. In high doses spermin prevents stimulating effect of hypothermia on respiration and reduces the increase in calcium capacity.

Key Words: *hypothermia; spermin; mitochondria; oxidative phosphorylation*

Study of energetics of mitochondrial processes in deep hypothermia is a pressing problem of experimental biology, because the survival of cells under conditions of low body temperature is determined by the ratio between their requirement in energy and the capacity to produce this energy [3]. Therefore, tissue and cell survival in hypothermia can be attained through the mitochondrial system.

Polyamines stimulate oxidative phosphorylation and calcium transport in isolated mitochondria *in vitro* [2,5,7]. We investigated the effect of spermin in normo- and hypothermia on oxidative processes in isolated rat liver mitochondria.

MATERIALS AND METHODS

Experiments were performed on Wistar rats of both sexes weighing 250-350 g. The animals were divided into the following groups: intact controls, Nembutal narcosis (50 mg/kg), intraperitoneal spermin in doses of 1, 5, and 10 mg/100 g, 19-20°C hypothermia in the presence of Nembutal narcosis and in combination with spermin injections in doses of 1, 5, and 10 mg/100 g.

Efficacy of narcosis was tested 40 min after intraperitoneal injection of Nembutal. Hypothermia was started after narcotization (5 min after narcotic injection) by surrounding the animal with ice pieces. Rectal temperature of 19-20°C was achieved in 40-45 min.

In experiments with polyamine, spermin was injected intraperitoneally in the required dose, the animal was cooled to a rectal temperature of 19-20°C, and then decapitated. In experiments without cooling, the animals were decapitated 40 min after spermin injection. Thus, the duration of spermin effect was the same in all experiments.

Mitochondria were isolated from the liver by differentiated centrifugation [6] in medium containing 0.3 M sucrose, 10 mM Tris-HCl buffer, and 1 mM EDTA.

Mitochondrial respiration was measured in a well by the polarographic method with Clark's electrodes in medium containing 0.05 M sucrose, 0.1 M KCl, 5 mM KH_2PO_4, 10 mM Tris-HCl buffer (pH 7.5) at

Department of Biophysics and Physiology, Daghestan State University, Makhachkala

0007-4888/98/0005-0465$20.00

Table 1. Respiration Rates (ng ADP/min×mg protein) and Calcium Capacity (μmol/$CaCl_2$/mg protein) in Isolated Rats Liver Mitochondria Exposed to Hypothermia and Spermin (*M±m*)

Animal status	V_1	V_2	V_3	V_4	V_5	V_3/V_2	V_3/V_4	ADP/O	Ca^{2+} capacity
Control (*n*=30)	11.2± 0.2	23.6± 0.5	105.0± 3.9	26.8± 1.0	152.5± 4.5	4.32± 0.08	3.70± 0.10	2.26± 0.03	0.278± 0.007
Narcosis (*n*=7)	10.6± 0.6	23.2± 0.6	91.6± 1.9	29.0± 1.2	134.5± 6.4	3.95± 0.06	3.19± 0.10	2.34± 0.06	0.368± 0.004
Hypothermia 20°C+narcosis (*n*=13)	13.0± 0.3**	36.5± 0.9***	131.9± 6.1***	39.9± 1.3***	243.5± 9.0***	3.40± 0.04***	3.15± 0.05**	2.09± 0.05*	0.451± 0.003***
Spermin, 1 mg (*n*=6)	11.3± 0.2	24.1± 0.8	101.6± 4.1*	25.6± 0.7	154.9± 3.5*	4.27± 0.20*	4.03± 0.10*	2.24± 0.03	0.272± 0.005***
Hypothermia 20°C+narcosis+ spermin, 1 mg (*n*=5)	11.8± 0.3	31.0± 1.2+++++	107.4± 3.7+	35.0± 1.0***	190.1± 5.7++++++	3.47± 0.10***	3.07± 0.10**	2.03± 0.05***	0.466± 0.009***
Spermin, 5 mg/100 g (*n*=5)	9.04± 0.4***	22.1± 0.5	93.6± 2.1**	24.3± 0.4	138.1± 3.1*	4.26± 0.06*	3.85± 0.06***	2.20± 0.02	0.253± 0.004**
Hypothermia 20°C+narcosis+ spermin, 5 mg/100 g (*n*=5)	12.2± 0.5+	28.9± 1.1++++++	118.3± 4.9++	31.0± 1.3+++++	178.0± 6.4++++	3.92± 0.07***	3.67± 0.09***	2.01± 0.03***	0.421± 0.005***
Spermin, 10 mg/100 g (*n*=6)	7.74± 0.5***	21.8± 0.9	73.9± 3.5***	23.4± 1.1	112.9± 4.6***	3.40± 0.06***	3.16± 0.04***	2.05± 0.02***	0.236± 0.005***
Hypothermia 20°C+narcosis+ spermin, 10 mg/100 g (*n*=3)	5.28± 0.4++++++	20.4± 1.0++++	68.2± 3.1++++++	24.3± 1.2+++	131.0± 5.3+++++	3.31± 0.04+++++++	2.82± 0.06+++++++	1.97± 0.03++++	0.407± 0.004++++

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ *vs.* the control; +$p<0.05$, ++$p<0.01$, +++$p0.001$ *vs.* hypothermia during Nembutal narcosis.

26°C [10]. The following parameters were measured after successive addition of substances: V_1, respiration rate on endogenic substrata; V_2, respiration rate after addition of succinate to a concentration of 4 mM; V_3, respiration rate after addition of ADP (200 μM); V_4, respiration rate after exogenous ADP was exhausted; and V_5, respiration rate after adding 2,4-dinitrophenol (0.1 mM). Based on the measured values, the respiratory control (V_3/V_2, V_3/V_4) and phosphorylation coefficient (ADP/O) were calculated. Calcium capacity of mitochondria was estimated by adding small portions of $CaCl_2$ to mitochondria respiring on succinate (without ADP) until respiration was irreversibly stimulated. Protein was measured by the method of Lowry [4].

RESULTS

Hypothermia stimulated mitochondrial respiration in all metabolic states, although the stages limiting the rate of oxygen consumption were different in these states (Table 1). The degree of oxidation contingency with phosphorylation changed negligibly. This may reflect the processes of thermal compensation in response to body temperature decrease. All hypothermic states were characterized by increased calcium capacity, reflecting the ability of mitochondria to uptake calcium from extramitochondrial space. Increase of this parameter in hypothermia may imply the necessity of maintaining low Ca concentrations in the cytoplasm at low temperature of tissue.

In high doses spermin depresses ADP- and dinitrophenol-stimulated respiration in a dose-dependent mode both in normo- and hypothermia. The cause of inhibitory effect of spermin is not clear. It may be due to spermin oxides, possessing a high biological activity even at low concentrations [1]. Like other polyamines, spermin stabilizes membrane structure *in vitro* [7].

Spermin slightly, but statistically significantly, decreased mitochondrial calcium capacity. In high doses it inhibited the stimulatory effect of hypothermia on mitochondrial respiration. Thus, spermin can be used for suppressing cell energetics, and this effect is potentiated by hypothermia.

REFERENCES

1. N. K. Berdinskikh and S. P. Zaletok, *Polyamines and Tumor Growth* [in Russian], Kiev (1987).
2. T. B. Votyakova, E. N. Bazhanova, and R. A. Zvyagil'skaya, *Biol. Membrany*, **9**, No. 4, 310-318 (1992).
3. E. Z. Emirbekov and S. P. L'vova, *Mechanisms of Biochemical Changes in Low Body Temerature* [in Russian], Rostov (1985).
4. J. H. Lowry, N. I. Rosenbrogh, A. L. Farr, and R. I. Randall, *J. Biol. Chem.*, **139**, 265-275 (1951).
5. A. N. Murphy, J. K. Keller, and G. Fiskum, *Ibid.*, **265**, No. 18, 10527-10534 (1990).
6. W. Schneider, *Adv. Enzymol.*, No. 2, 1-72 (1959).
7. F. Schuber, *Biochem. J.*, No. 1, 1-10 (1989).