# ACTION OF PANTHETIN ON METABOLISM IN MYOCARDIAL MITOCHONDRIA DURING DEEP HYPOTHERMIA

**L. Ya. Utno**

UDC 577.121:577.152.283

KEY WORDS: myocardium; mitochondria; panthetin; CoA; malate dehydrogenase.

The duration of possible deep hypothermia (20 ± 2°C) is limited, for if this method is used for a long time irreversible changes develop in the myocardial mitochondria. These changes are manifested primarily as disturbance of the structure of some mitochondria [2] and accumulation of peroxidation products [5], which have an inhibitory action on energy formation in the myocytes. We found that preliminary administration of panthetin prevents the development of destructive changes in the myocardial mitochondria during hypothermia [1] and restores normal lipid peroxidation [6]. Considering that the metabolic activity of panthetin is effected mainly by its biotransformation into CoA [3], in the investigation described below we studied the action of panthetin on the formation of total and free CoA, and LCA-CoA, and on activity of CoA-dependent dehydrogenases and malate dehydrogenase in the mitochondria and also on the content of high-energy compounds in the myocardium.

## EXPERIMENTAL METHOD

Experiments were carried out on 83 anesthetized male Wistar albino rats weighing 190-220 g. Anesthesia was induced 30 min before cooling (with a mixture of 10% thiopental solution, 20% γ-hydroxybutyrate solution, and 0.1% atropine solution and water in the ratio 1:1.6:0.4:1) in a dose of 0.5 ml/100 g body weight; morphine was injected separately in a dose of 0.5 mg/100 g body weight. Anesthesia was carried out in the manner most frequently used during hypothermia in heart surgery.

The schedule of the experiments was as follows: group 1) control, group 2) deep hypothermia, group 3) deep hypothermia preceded by injection of panthetin in a dose of 50 mg/kg 2.4, 6, and 24 h previously. In the investigations of the action of panthetin, a commercial preparation from the firm Daiichi was used. The animals of groups 2 and 3 were cooled by surrounding them with finely crushed ice at the rate of 0.6-0.7°C/min to a rectal temperature of 20 ± 2°C, after which the animals were kept under these conditions for 40 min.

Mitochondria were isolated by differential centrifugation [7]; activity of pyruvate dehydrogenase was determined by the method in [8], 2-oxoglutarate dehydrogenase by the method in [1], the content of LCA-CoA by the method in [10], acetyl-CoA synthetase activity by the method in [9], and the content of MDA and creatine phosphate by methods described previously [4].

## EXPERIMENTAL RESULTS

It will be clear from the results in Table 1 that during deep hypothermia the total content of CoA in the myocardium is reduced on account of a decrease in both free CoA and acetyl-CoA. Meanwhile the content of long-chain acyl groups of CoA is significantly increased.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 577-578, June, 1991. Original article submitted November 27, 1990.

TABLE 1. Content of Long-Chain Acyl Groups of CoA, Acid-Soluble Total and Free CoA in Myocardium during Deep Hypothermia

| Parameter studied | Control | Deep hypothermia | Deep hypothermia + panthetin |
|---|---|---|---|
| Content of long-chain CoA, mmoles/mg protein | 13,3±3,0 | 25,0±5 | 9,7±1,7 |
| *p* | | <0,05 | |
| Content of acid-soluble CoA, mmoles/g | 184±14 | 121±7 | 197±12 |
| *p* | | <0,01 | |
| Content of free CoA, mmoles/g | 118±10 | 69±8 | 109±6 |
| *p* | | <0,001 | |
| Content of total CoA | 223±8 | 193±4 | 282±4 |
| *p* | | <0,05 | |
| Acetyl-CoA-synthetase activity in cytosol, mmoles acetyl-CoA/mg protein/min | 2,44±0,21 | 2,34±2,8 | 3,15±0,22 |

TABLE 2. Action of Panthetin on Pyruvate Dehydrogenase and α-Ketoglutarate Dehydrogenase Activity in Mitochondria and on ATP in Myocardium during Deep Hypothermia

| Index of activity of enzymes | Control | Deep hypothermia | Deep hypothermia + panthetin |
|---|---|---|---|
| Pyruvate dehydrogenase activity of mitochondria, mmoles reduced ferricyanide | 42±6 | 68±11 | 106±8 |
| Activity of mitochondrial 2-oxoglutarate dehydrogenase, mmoles reduced ferricyanide/mg protein/min | 84±14 | 107±11 | 137±11 |
| Malate dehydrogenase activity in myocardial mitochondria, μmole/mg protein/min | 2,24±0,097 | 1,86±0,12 | 2,65±0,22 |
| ATP content in myocardium, μ moles/g tissue | 3,53±0,26 | 3,23±0,16 | 4,07±0,16 |
| Creatine phosphate content, μmoles/g tissue | 1,7±0,2 | 1,73±0,32 | 2,6±0,41 |

Injection of panthetin increased the CoA content in the myocardial cytosol on account of transfusion of CoA formed as a result of hydrolysis of the preparation in the plasma. By virtue of the presence of SS-groups the hydrolysis products formed protein complexes, which gradually released CoA.

After preliminary injection of panthetin in a dose of 50 mg/kg a considerable increase in activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase was found in the myocardial mitochondria (Table 2). Evidently after injection of panthetin sufficient of the free CoA was formed to induce an increase in pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase activity. As a result of activation of the pyruvate dehydrogenase complex, the increase in content of acetyl-CoA (Table 1) acted as a signal for an increase in the quantity of oxaloacetate to provide for the more rapid oxidation of malate in the Krebs' cycle. As Table 2 shows, preliminary injection of panthetin caused an increase in malate dehydrogenase activity in the myocardial mitochondria by 42% The increase in malate dehydrogenase activity in the mito-

TABLE 3. Action of Panthetin on Phospholipid Composition of Mitochondria of Heart (in %) and on MDA Formation (in mmoles/mg protein) in Mitochondria

| Parameter studied | Control | Deep hypothermia | Deep hypothermia + panthetin | $p$ |
|---|---|---|---|---|
| Phosphatidylcholine | 29,5±0,9 | 30,6±2,3 | 29,0±1,1 | <0,05 |
| Phosphatidylethanolamine | 23,5±2,1 | 24,4±0,7 | 27,0±0,9 | <0,05 |
| Phosphatidylinositol | 15,1±2,0 | 11,4±1,8 | 13,0±0,8 | <0,05 |
| Cardiolipin | 18±0,9 | 18,1±1,6 | 18,4±1,1 | >0,05 |
| Sphingomyelin | 13,6±1,8 | 15,5±2,3 | 12,6±1,2 | >0,05 |
| MDA | 1,02±0,081 | 2,36±0,19 | 1,31±0,26 | <0,05 |

chondria was evidently determined by the increase in malate concentration. Replenishing the malate reserves as a result of reductive carboxylation of pyruvate is catalyzed by the pyruvate dehydrogenase enzyme complex, whose activity was increased by 2.5 times after injection of panthetin. A 1.7-fold increase in 2-oxoglutarate dehydrogenase activity also was found in the experiments. Activation of 2-oxoglutarate dehydrogenase catalyzes the formation of succinyl-CoA, which can phosphorylate guanosine diphosphate.

The terminal phosphate group of this compound, whose formation is catalyzed by succinyl-CoA-synthetase, phosphorylates ADP. As a result, ATP is formed. These data explain the significant increase in ATP in the rat myocardium in experiments with deep hypothermia after injection of panthetin. An increase in the creatine phosphate content also was found (Table 2).

Consequently, the injected panthetin, through biotransformation into CoA, and its acyl derivatives have an effector action on dehydrogenases and carboxygenases during hypothermia.

Activation of these enzyme complexes preserves the activity of reactions of formation of high-energy compounds in the myocardium during hypothermia.

In our experiments injection of panthetin caused an increase of 17% in synthesis of phosphatidylethanolamine, and reduced activity of formation of lipid peroxidation products in the mitochondria (Table 3).

CoA and its acyl derivatives are known to be effectors of synthetases [4] and the acetyl-CoA/CoA-SH ratio is a regulator of the direction of transport of the acetyl radical from the mitochondria into the cytosol for lipid synthesis. Panthetin, as a precursor of CoA, evidently plays a specific role in the regulation of biosynthesis of phosphatidylethanolamine, which together with phosphatidylcholine is a basic component of the mitochondrial and plasma membrane. Changes in the phosphitidylethanolamine concentration in the mitochondrial membranes and the increase in content of high-energy compounds are evidently factors inhibiting activation of mitochondrial lipid peroxidation during deep hypothermia.

It can be concluded from the results of these experiments that panthetin has an adaptogenic action, by promoting adequate changes in function and structure of the myocardial mitochondria during deep hypothermia.

Consequently, during deep hypothermia panthetin has not only the action of a coenzyme, but also an effector action on metabolism in the myocardial mitochondria.

## LITERATURE CITED


1. A. I. Biryukov, N. B. Tarusov, S. G. Amontov, et al., Bioorg. Khim., **11**, No. 5, 598 (1985).
2. A. R. Dalmane, L. Ya. Utno, M. Yu. Anshelevich, and V. A. Rotabyl'skii, Izv. Akad. Nauk Latv. SSR, No. 4, 101 (1988).
3. L. Ya. Utno, Z. O. Lipsberga, A. A. Silova, et al., Byull. Éksp. Biol. Med., No. 11, 558 (1989).
4. J. R. Neely, J. D. Robishaw, and T. C. Vary, in: Myocardial Metabolism [Russian translation], Moscow (1983), pp. 71-83.
5. L. Ya. Utno, Izv. Akad. Nauk Latv. SSR, No. 7, 78 (1989).
6. L. Ya. Utno and V. A. Korzan, Proceedings of the 3rd International Symposium on Metabolism, Structure, and Function of the Heart Cell [in Russian], Moscow (1986), p. 91.
7. F. Z. Crane, A. V. Glenn, and D. E. Green, Biochim. Biophys. Acta, **22**, 475 (1956).

8. G. I. Grebler, J. Biol. Chem., **234**, No. 8, 3112 (1961).
9. P. K. Tublus and P. V. Gauland, Methods Enzymol., **12**, 535 (1969).
10. I. P. Williamson and B. E. Corney, Methods Enzymol., **13**, 434 (1969).

# MEASUREMENT OF THE INTRACELLULAR $Ca^{2+}$ CONCENTRATION IN MACROPHAGES AND THE EFFECT OF PLATELET ACTIVATION FACTOR

**V. G. Pinelis, D. V. Zagulova, A. A. Galkin, M. B. Baskakov, and Kh. M. Markov**

UDC 616.155.33-008.924.1-074

KEY WORDS: $Ca^{2+}$, platelet activation factor, macrophages, fluorescent indicator

The basic functions of macrophages, namely chemotaxis, degranulation, phagocytosis, and the "oxygen burst" [1, 11, 14] determine their key role in inflammatory reactions. Stimulated macrophages, in turn, secrete mediators of inflammation, namely prostaglandins, leukotrienes, platelet activation factor (PAF), superoxide anions, and interleukin-1 [12, 13]. An important role in these processes is played by $Ca^{2+}$, namely by changes in the intracellular concentration of this cation. For instance, an increase in $[Ca^{2+}]_i$ in macrophages, produced by means of ionophore $A_{23187}$, causes the appearance of tumoricidal activity and the secretion of tumor necrotic factor (TNF), expression of various antigens on the membrane surface, and an "oxygen burst," whereas antagonists of calcium ions and calmodulin inhibit activity of the macrophages [7, 9, 15]. There is some evidence in the literature [3, 8, 10] of a change, through the influence of $[Ca^{2+}]_i$ agonists in macrophages stimulated by sodium thioglycollate, although definite functional differences are known in the responses of resident and stimulated macrophages to the action of biologically active substances [1, 3]. It must also be pointed out that in [3], $[Ca^{2+}]_i$ was determined with the aid of the fluorescent probe quin-2, which has definite shortcomings (toxicity, low quantum yield, lower dissociation constant).

In the present investigation a new $Ca^{2+}$-sensitive fluorescent indicator fura-2 was used to study changes in $[Ca^{2+}]_i$ in resident and activated macrophages under the influence of PAF. PAF is a natural mediator of inflammatory reactions, of phospholipid nature, synthesized in many cells including macrophages (see above). In turn, mechanisms whereby the released PAF stimulates macrophages have not been finally established, and data in the literature are contradictory [3, 8].

## EXPERIMENTAL METHOD

Resident and stimulated macrophages obtained from mice killed by cervical dislocation were used. In the latter case, 4 days before isolation of the cells the animals were given an intraperitoneal injection of 1.5 ml of a 2% solution of sodium thioglycolate; cells from peritoneal washings, obtained by injection of 5-7 ml of Hanks' solution (pH 7.35) into the peritoneal cavity, were sedimented on coverslips measuring 7 × 15 mm in order to obtain a monolayer, which was recorded under the microscope, or cell suspensions were prepared.

Research Institute of Pediatrics, Academy of Medical Sciences of the USSR. A. V. Vishnevskii Institute of Surgery, Moscow. Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR M. Ya. Studenikin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 579-581, June, 1991. Original article submitted October 2, 1989.

 0007-4888/91/0006-0752$12.50 