Comparative Study of Cardioprotective Effects of Uridine-5'-Monophosphate and Uridine-5'-Triphosphate during the Early Periods of Acute Myocardial Ischemia

V. V. Bul'on, I. B. Krylova, O. M. Rodionova, E. N. Selina, N. R. Evdokimova, and N. S. Sapronov

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In experiments on rats, uridine-5'-monophosphate and uridine-5'-triphosphate reduced the intensity of anaerobic glycolysis and preserved glycogen stores and creatine phosphate balance during the first 60 min after occlusion of the left coronary artery. However, the energy-protective effect of uridine-5'-triphosphate developed 15 min later than the effect of uridine-5'-monophosphate. Uridine-5'-monophosphate, but not uridine-5'-triphosphate, reduced *T* wave amplitude on ECG and decreased the volume of ischemic injury to the myocardium.

Key Words: *myocardium; ischemia; energy metabolism; uridine-5'-monophosphate; uridine-5'-triphosphate*

Hypoxia plays a key role in the pathogenesis of ischemic injury to the myocardium. Oxygen deficiency in tissues leads to suppression of aerobic energy formation in tissues, uncoupling of oxidative phosphorylation, energy deficit, activation of free radical oxidation, and changes in Ca^{2+} homeostasis, which leads to impairment of membrane integrity and irreversible damage to cardiomyocytes [1,6]. Therefore, drugs reducing these metabolic shifts should be rationally used in the therapy of coronary disease. Substances acting at the level of endogenous regulators of cardiac metabolism and function are particularly interesting in this respect [3].

We compared the cardioprotective effects of uridine nucleotides, uridine-5'-monophosphate (UMP) and uridine-5'-triphosphate (UTP), characterized by a wide spectrum of pharmacological effects [10].

Department of Neuropharmacology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** sns@iem.spb.ru. Bul'on V.V.

MATERIALS AND METHODS

Experiments were carried out on 130 male Wistar rats (250-300 g). Myocardial ischemia was induced by ligation of the descending branch of the left coronary artery (LCA) at the level of the lower edge of the left auriculum under conditions of artificial ventilation. The animals were narcotized with sodium ethaminal (50 mg/kg). Uridine nucleotides (UMP and UTP; ICN) in a dose of 30 mg/kg were injected into the jugular vein directly after LCA occlusion. Control rats received saline.

The effects of uridine nucleotides on the content of lactate, pyruvate, glycogen, and creatine phosphate were studied 15, 30, and 60 min after LCA ligation [2,7]. The severity of ischemic damage to the myocardium was evaluated 60 min after LCA occlusion by *T* wave amplitude on ECG and

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by the index of ischemic injury to the myocardial tissue [11,12].

The data were processed statistically using Student's *t* test.

RESULTS

The concentration of lactate in rat heart 15, 30, and 60 min after LCA occlusion increased by 237, 311, and 211%, respectively, in comparison with intact animals (Fig. 1). No appreciable changes in pyruvate content were detected during these periods of ischemia. The lactate/pyruvate ratio reflecting the intensity of glycolytic or aerobic pathway of energy formation increased from 11 to 43, 49, and 34, respectively.

Under conditions of impaired coronary circulation, when delivery of free fatty acids and glucose with the blood is reduced, the heart utilizes glycogen as the energy substrate; glycogen stores are small and therefore are rapidly exhausted. The greatest drop (52%) of glycogen content was observed by the 30th minute of ischemia. This was paralleled by a decrease in the content of creatine phosphate, which was also most pronounced during the 30th min of ischemia (61%).

These data suggest that LCA occlusion significantly disturbed energy metabolism in the heart, which manifested in reduced intensity of aerobic pathway of the substrate oxidation, activation of glycolysis, deficit of glycogen reserve, and reduced content of creatine phosphate.

Disturbances in energy metabolism and energy deficiency paralleled by activation of free radical oxidation and suppression of endogenous antioxidant systems are the main cause of ischemic

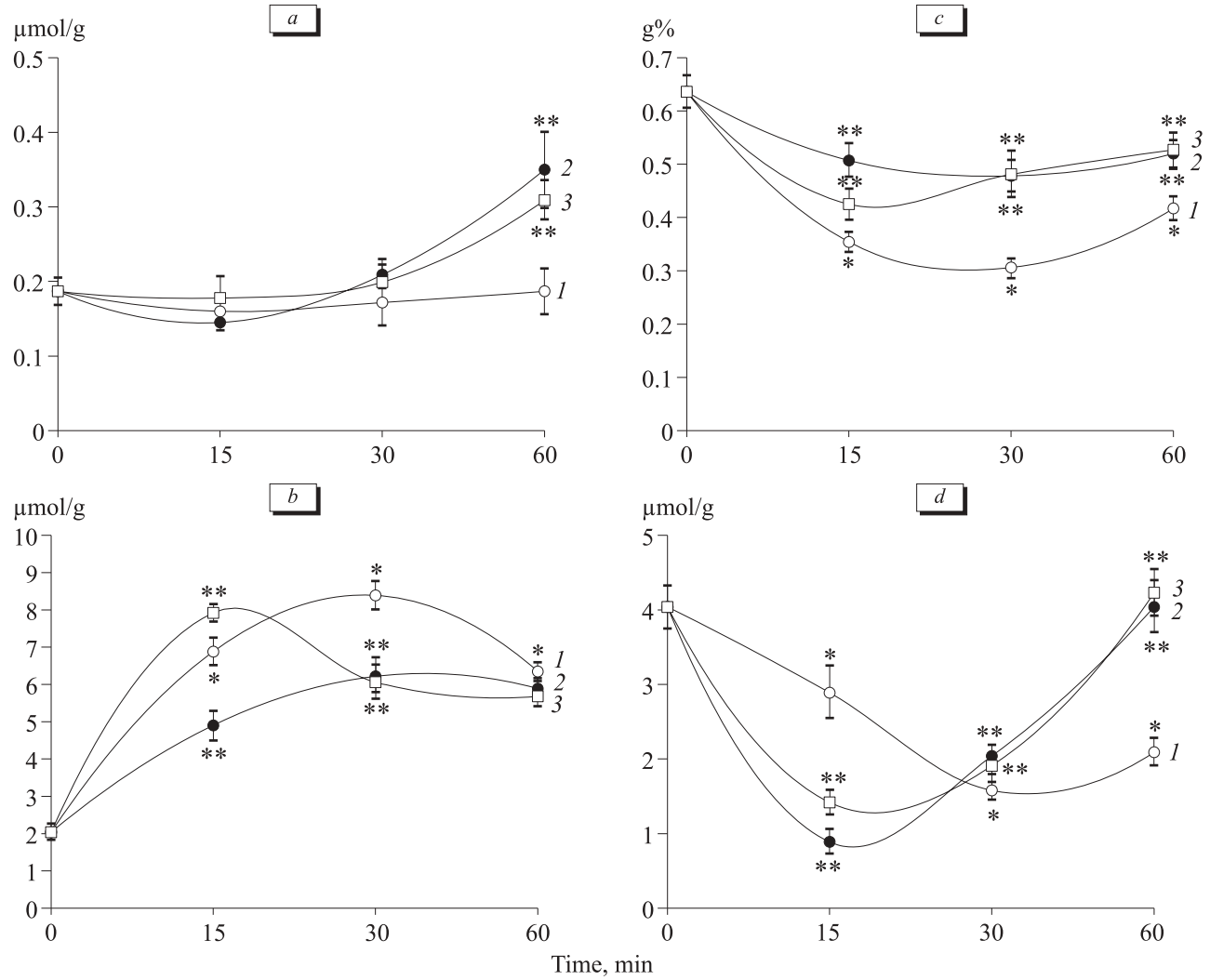

Fig. 1. Effects of UMP and UTP on parameters of energy metabolism in ischemic myocardium. *a*) pyruvate; *b*) lactate; *c*) glycogen; *d*) creatine phosphate. *1*) control (ischemia); *2*) UMP; *3*) UTP. **p*<0.05 compared to intact animals (before treatment); ***p*<0.05 compared to the control.

damage to cardiomyocytes [2]. The amplitude of *T* wave increased by 2.8 times in comparison with the initial value as soon as 3 min after LCA occlusion and this increase persisted throughout the entire observation period (60 min), which indicated ischemic disorders in the myocardium. Sixty minutes after LCA ligation, the zone of ischemic injury occupied 30-50% of the left-ventricular volume. The index of ischemic injury to the myocardium was 1.27±0.04 (Table 1).

Exogenous UMP can affect metabolic processes after its transformation into uridine, uridine-5'-diphosphate (UDP), UTP or UDP-glucose in cells [10]. Extracellular UTP is not transported through membranes, and therefore it can affect cell metabolism only after dephosphorylation to UMP or uridine with their subsequent intracellular transformations. The rate of uridine incorporation into intracellular pool of uridine compounds increases significantly under conditions of reduced coronary flow [5]. Uridine-5'-triphosphate exhibits its effects also at the receptor level [13].

The protective effects of UMP and UTP on the cardiac energy metabolism were clearly seen 60 min after LCA occlusion. After injection of UMP, the lactate/pyruvate ratio decreased to 34, 30, and 17 *vs.* 43, 49, and 34 in the control, which reflected reduced intensity of glycolysis and increased contribution of aerobic formation of macroergic compounds during all periods of occlusion. In contrast to UMP, UTP decreased the lactate/pyruvate ratio starting from the 30th min of ischemia (to 45, 30, and 18).

Both nucleotides preserved glycogen depots essential for the synthesis of the glycolytic fraction of ATP playing a unique role in the maintenance of functional and structural integrity of cardiomyocytes during early periods of ischemia [3]. The content of glycogen approached its level in intact rats after 60 min of the experiment, which could be due to stimulation of glycogenesis. It is known that metabolism of uridine compounds yields UDP-glucose, a substrate for glycogen synthesis [4].

In animals receiving UMP and UTP, the content of pyruvate in the heart on the 60th minute of LCA occlusion increased by 87 and 65%, respectively, in comparison with intact animals. Pyruvate entering the Krebs cycle with subsequent mitochondrial oxidation promotes the maintenance of energy balance in cardiomyocytes. The content of creatine phosphate playing an important role in energy supply of myocardial contractions and regulation of Ca^{2+} flows in animals receiving UMP and UTP on the 60th minute of LCA occlusion did not differ from that in intact animals.

The content of creatine phosphate in rats receiving UMP and UTP by the 15th minute of coronary occlusion was lower than in the corresponding control and slightly increased only by the 30th minute. This fact can be explained by a positive inotropic effect of nucleotides [5]. The increase of myocardial contractile function in response to UMP and UTP injection is paralleled by increased consumption of cytoplasmic creatine phosphate during the earliest period of ischemia, which is not compensated by its transport from mitochondria because of ATP-ADP translocase inhibition (this enzyme is functionally conjugated with mitochondrial creatine kinase). Translocase is very sensitive to O_2 deficit: its activity drops by 80% during 15-min ischemia.

The energy-protecting effect of UMP can be associated with the formation of β-alanine during metabolism of this nucleotide. As is known, β-alanine is a component of acetyl-CoA (as a fragment of pantothenic acid) participating in redox processes and its formation maintains energy potential of cells [10].

In addition, exogenous uridine nucleotides replenishing the intracellular UDP pool can activate ATP-dependent mitochondrial K^+-channels [12] playing the key role in antihypoxic protection of the myocardium [8,9,14]. Presumably, changes induced by activation of these channels limit mitochondrial damage and prevent uncoupling of oxidative phosphorylation, reduction of ATP synthesis, and energy imbalance in ischemic cardiomyocytes.

TABLE 1. Effects of UMP and UTP on *T* Wave Amplitude and Index of Ischemic Injury to the Myocardium 60 min after LCA Occlusion in Rats (*M*±*m*; *n*=8-10)

Group	*T* wave amplitude, mV		Index of ischemic injury
	before occlusion	60 min after occlusion	
Control	0.19±0.01	0.53±0.04	1.27±0.04
UMP	0.17±0.02	0.34±0.02*	0.30±0.10*
UTP	0.20±0.02	0.64±0.09	0.90±0.11

Note. *$p<0.05$ compared to the control.

It is known that substances with energy-protecting effects protect cardiomyocytes from ischemia [2]. The results of this study demonstrate that UMP reduced *T* wave amplitude and zone of myocardial ischemia by the 60th minute of LCA occlusion by 36 and 77%, respectively, in comparison with the control, which indicates antiischemic activity of this drug manifesting during the early period of acute myocardial ischemia [12].

In contrast to UMP, UTP exhibited no antiischemic effect, which seems paradoxical, because energy-protective effect of UTP was expected to increase of antiischemic resistance of cardiomyocytes. Moreover, UTP via P_{2U} receptors on endothelial cells stimulates blood flow in the heart [5], thus determining the antianginal effect of this compound. However, apart from endothelial P_{2U} receptors, UTP interacts with P_{2U} receptors on cardiomyocytes. Stimulation of these receptors increases the content of intracellular Ca^{2+}, which can damage cardiomyocytes [4]. It can be hypothesized that at the early period of ischemia this receptor effect of UTP masks all positive aspects of its effect on metabolism and blood supply to the myocardium.

Hence, UMP and UTP exhibited protective effects on myocardial energy metabolism during the acute period of ischemia. The energy-protecting effect of UTP was delayed by 15 min in comparison with the analogous effect of UMP. UMP, but not UTP, exhibited antiischemic effect during the early period of acute myocardial ischemia. The combination of energy-stabilizing and antiischemic effects offer UMP as a potential cardioprotective drug.

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