Effect of Uridine Derivatives on Myocardial Stunning during Postischemic Reperfusion of Rat Heart

N. S. Sapronov, V. V. Eliseev, and O. M. Rodionova

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Uridine and uridine-5'-monophosphate prevent myocardial stunning during postischemic reperfusion of isolated rat heart. Uridine-5'-diphosphate does not prevent postischemic myocardial dysfunction, while uridine-5'-triphosphate aggravates it.

Key Words: *uridine; uridine nucleotides; myocardial stunning*

Myocardial stunning (postischemic dysfunction of the myocardium) is the major manifestation of postischemic reperfusion syndrome. Ischemic myocardium remains hypokinetic or even akinetic after restoring the coronary blood flow [2,7,11]. The main causes of stunning is ATP deficiency, LPO activation in cell membranes, calcium overload and contracture of cardiomyocytes, and disturbed excitation-contraction coupling.

Glycolysis activators can be used to prevent reperfusion syndrome. On the one hand, glycolysis is the major energy source during ischemia, and on the other hand, it provides ATP for myocardial contraction and function of the calcium pump [3]. These activators are uridine and uridine nucleotides: uridine-5'-monophosphate (UMP), uridine-5'-diphosphate (UDP), and uridine-5'-triphosphate (UTP). Being precursors of uridinediphosphoglucose (UDPG, a cofactor of glycogen synthesis), these substances play an important role in the maintenance of myocardial contractile activity. During ischemia, glycogenolysis is the only mechanism of anaerobic ATP synthesis, because glucose transport to the heart is restricted. However, glycogen stores in cardiomyocytes are rapidly depleted. The glycogen content in perfused rat heart after 30-min total ischemia decreased by 60% [12]. The following 30-min reperfusion did not restore the glycogen level. Despite sufficient glucose concentration in the perfusate, the left ventricle developed pressure (LVDP) to the end of the reperfusion period was only 28% of the initial value. Depletion of glycogen stores is accompanied by a decrease in the content of UTP and UDPG in the myocardium [5,6]. However, addition of 5-50 μmol/liter uridine to the perfusate during postischemic reperfusion markedly increased the content of UTP, UDPG, and glycogen in isolated rat heart against the background of accelerated uridine incorporation into cardiomyocytes. Our aim was to evaluate the effects of uridine, UMP, UDP, and UTP on contractile activity of the left ventricle and coronary blood flow (CBF) during reperfusion of isolated rat heart after a 30-min total ischemia.

MATERIALS AND METHODS

The study was carried out on Langendorff-perfused hearts isolated from albino random-bred male rats weighing 250-280 g. The chest was opened under ether narcosis, the heart was isolated, washed with cold (4°C) Krebs—Henseleit solution, and connected to a perfusion system. Perfusion was performed with Krebs—Henseleit solution containing (in mM): 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.6 $MgSO_4$, 25.0 $NaHCO_3$, 0.5 Na-EGTA, and 5.5 glucose, saturated with 95% O_2 and 5% CO_2 mixture (37°C; pH 7.4), and delivered at a constant pressure of 97 cm H_2O. A small latex balloon connected through a catheter to a EMT-746 semiconductor pressure transducer (Siemens-Elema) was inserted into the left ventricle. LVDP, end-diastolic pressure (EDP), and the maxi-

Department of Neuropharmacology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** sns@iem.spb.ru. Sapronov N. S.

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mum contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) rates were determined under isovolumic conditions. The initial EDP was 10 mm Hg. Periodically, coronary blood flow (CBF) was evaluated by measuring the volume of perfusate flowing through the heart during 1 min. After 15-min stabilization perfusion was

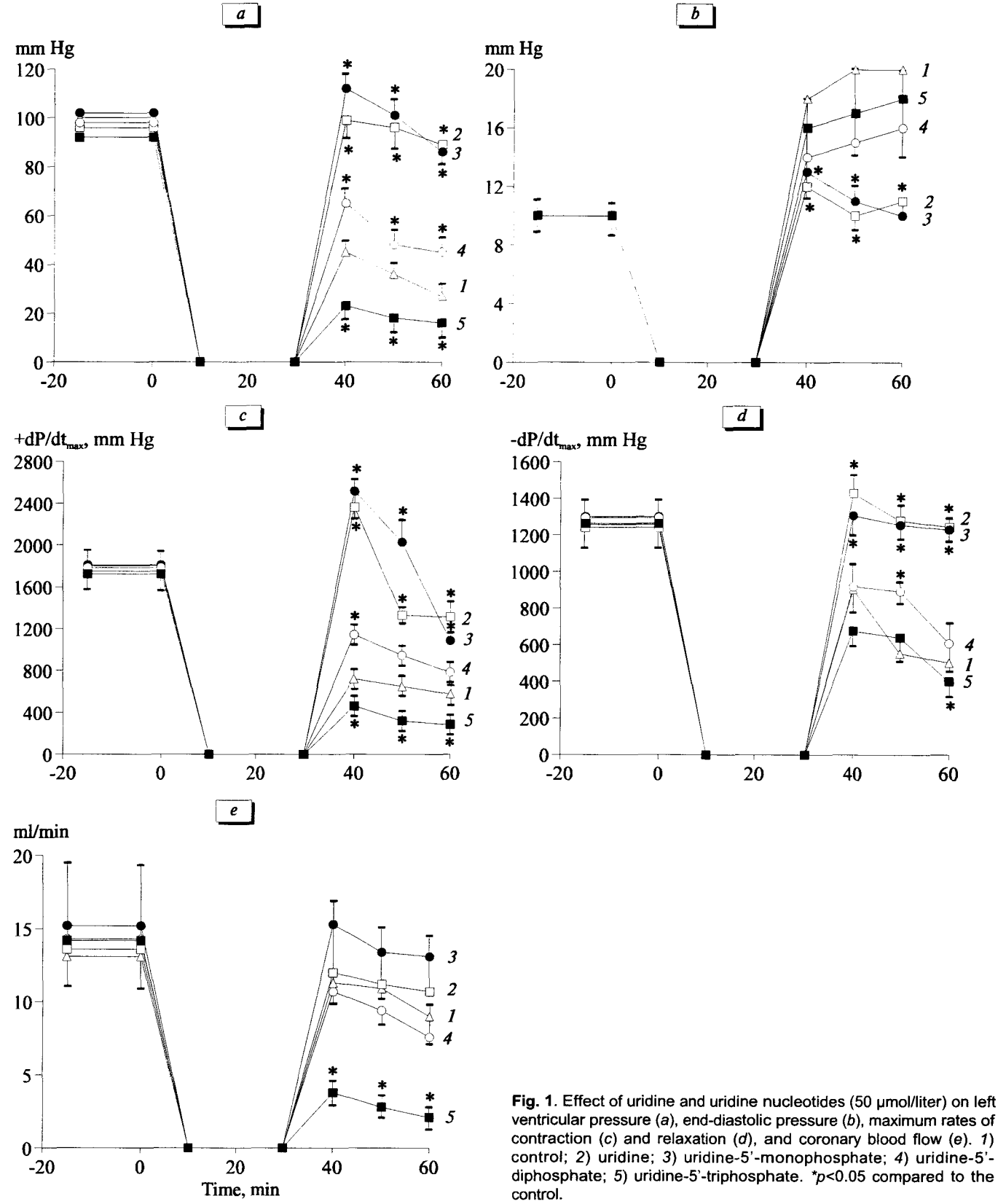

Fig. 1. Effect of uridine and uridine nucleotides (50 μmol/liter) on left ventricular pressure (*a*), end-diastolic pressure (*b*), maximum rates of contraction (*c*) and relaxation (*d*), and coronary blood flow (*e*). *1*) control; *2*) uridine; *3*) uridine-5'-monophosphate; *4*) uridine-5'-diphosphate; *5*) uridine-5'-triphosphate. *$p<0.05$ compared to the control.

stopped and 30-min total ischemia of the myocardium was modeled (temperature of the myocardium was maintained at 37°C). Then the heart was reperfused during 30 min either with control solution (control group) or with modified Krebs—Henseleit solution containing uridine, UMP, UDP, or UTP (Reanal) in concentration of 50 μmol/liter (the test groups). Each group comprised 8 hearts. The data were analyzed statistically using Microcal Origin 3.5 software. The differences were significant at $p<0.05$.

RESULTS

In the control group, postischemic reperfusion of the hearts was accompanied by a decrease in LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$ by 55-73%, 60-68% and 33-60%, respectively, in comparison with ischemia. At the same time, EDP increased 2-fold, while CBF decreased by 14-20% (Fig. 1). These changes confirmed the development of myocardial stunning.

Uridine added to the perfusate during reperfusion promoted recovery of myocardial inotropic function and improved its relaxation, but no increase in CBF was observed. UMP produced a more pronounced effect: being added to the perfusate, it normalized contractile activity after 20-min reperfusion: $+dP/dt_{max}$ surpassed that in the uridine group by 38% ($p<0.05$), contracture did not develop, and CBF returned to normal. UDP slightly increased LVDP, $+dP/dt_{max}$, and $-dP/dt_{max}$, decreased EDP, and virtually did not change CBF compared to those in the control group. The effects of UTP were negative: it impaired myocardial contractility and relaxation and markedly decreased CBF.

Therefore, only uridine and UMP prevented the development of myocardial stunning during postischemic reperfusion. UDP was ineffective, while UTP potentiated ischemia-reperfusion damage to the heart.

Incorporation of exogenous uridine into rat heart increases during postischemic reperfusion in comparison with the preischemic period [6]. Simultaneously, the content of UTP and UDPG (precursor of myocardial glycogen) increased. Restoration of glycogen stores in the presence of uridine, and the recovery of glucose transport to ischemic myocardium ensure more adequate energy supply to the heart. It is especially true for the glycolytic fraction of ATP, which provides the work of Ca^{2+}-pump and diastolic relaxation of the heart. The efficiency of uridine in moderating the postischemic disturbances of myocardial relaxation and contraction is known to be the same as that of increased glucose concentration in the perfusate [4]. This fact was corroborated in our experiments: uridine normalized $-dP/dt_{max}$ and EDP. Correspondingly, LVDP and $+dP/dt_{max}$ increased, and myocardial stunning were eliminated.

UMP produced similar effects. Exogenous nucleosides synthesized from the corresponding nucleotides under the control of sarcolemmic ecto-5'-nucleosidases are more rapidly utilized by the myocardium than native nucleosides [13], and ensure more intensive glycogen resynthesis. Exogenous UDP and UTP can be dephosphorylated to uridine with subsequent transport of nucleoside across the membrane and its conversion into UTP and UDPG. However, it should be taken into consideration that UDP and, in particular, UTP are the agonists of purine (pyrimidine) P_{2U} receptors located on the outer surface of cardiomyocytes and endothelial cells [8,9] and coupled with phospholipase via G-mediated mechanisms. Activation of these receptors leads to accumulation of inositol-1,4,5,-triphosphate and Ca^{2+} in cardiomyocytes, which aggravates "calcium paradox" and myocardial stunning during postischemic reperfusion.

On the other hand, UTP and, to a lesser degree, UDP acting on P_{2U} receptors on the endothelium of coronary vessels induce their dilation, which can be accompanied by reoxygenation and activation of LPO (oxygen paradox) with pronounced widening of nonperfusion area and aggravation of Ca^{2+} overload in the cardiomyocytes. Similar conditions characterize, for example, postischemic reperfusion of rat and rabbit hearts with solutions containing purine nucleoside adenosine producing a pronounced dilation of coronary arteries via vascular purine (adenosine) $P_1(A_2)$-receptors [1,10].

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