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# METABOLIC EFFECTS OF INOSINE AND URIDINE IN RABBIT HEARTS AND RAT SKELETAL MUSCLES

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**Abstract**—Because of their cardiotonic properties and their close relations with tissue energetic stores, we studied the inotropic and metabolic effects of inosine and uridine in isolated perfused rabbit hearts and incubated rat diaphragm muscles. Inosine and uridine in concentrations of $10^{-5}$ M increased myocardial contractility, glucose uptake, myocardial glycolysis and the breakdown of ATP in rabbit hearts. Uridine also increased glycogen content, most probably due to a stimulation of glycogen synthesis by this nucleoside. Neither nucleoside had an effect on myocardial lipolysis. Similarly, inosine in concentrations of $10^{-4}$ to $10^{-6}$ M stimulated glucose uptake and the rate of glycolysis in incubated rat diaphragm muscles. On the other hand, uridine in concentrations of $10^{-4}$ to $10^{-6}$ M stimulated glucose uptake and the content of glycogen in diaphragm muscles. However, no effect of uridine on glycogen content was observed when these muscles were incubated in Krebs bicarbonate medium without glucose. The inotropic and metabolic effects of inosine, but not of uridine, were abolished in isolated rabbit hearts treated with $10^{-5}$ M propranolol. Despite this inhibition, it seems unlikely that the action of inosine and uridine is mediated by endogenous catecholamines. On the other hand, their metabolic effects are similar to those of cardiac glycosides.

Among the degradative products of adenine and uracil nucleotides, inosine and uridine have been found to be especially effective in increasing myocardial contractility in mammalian hearts [1, 2]. The elevated tension of ventricular muscle responding maximally to inosine was further increased by the addition of uridine, suggesting that these compounds act by different mechanisms [1]. It has been further observed that the inotropic effect of inosine, but not of uridine, is inhibited in rabbit hearts by reserpine [3]. Since reserpine interferes with the metabolism of catecholamines in the heart [4], it has been suggested that endogenous catecholamines mediate the inotropic effects of inosine, while the action of uridine seemed to be independent of adrenergic mechanisms [3].

Inosine is a degradative product of high energy phosphate compounds, and has been recovered in high quantities when the breakdown of ATP is stimulated, e.g. in hypoxia or ischemia [5, 6]. On the other hand, uridine is a precursor of coenzymes or metabolites involved in the synthesis of glycogen, e.g. UTP, UDP and UDP-glucose [7]. Despite their close relations with the metabolism of important energetic stores, as well as their inotropic properties, metabolic effects of inosine and uridine in the myocardium have not yet been studied.

The purpose of the present investigation was therefore 2-fold: (1) to study the metabolic effects of inosine and uridine in the isolated perfused rabbit heart, and (2) to clarify the role of endogenous catecholamines in the mechanism of action of inosine and uridine by studying their effects in rabbit hearts treated with propranolol, a beta-receptor inhibitor. The metabolic effects of inosine and uridine were also studied in rat diaphragm muscle, a tissue in which the release of catecholamines is unlikely to be an important factor.

## METHODS

*Experiments with isolated perfused rabbit hearts.* Albino rabbits of both sexes, 6–8 weeks old, were maintained under standard conditions and allowed free access to food and water. The isolated rabbit heart was prepared in the following way: a rabbit was first anesthetized by the intraperitoneal administration of 30 mg/kg of sodium pentobarbital and tied in a supine position. The animal was mechanically ventilated with room air by means of an endotracheal tube and a rodent respirator. After the chest of the animal was opened, the heart and all connected vessels were isolated from surrounding tissues. A plastic catheter of a small diameter was inserted into one of the branches of the aortic arch, tied in the position, and 1-ml Lipo Hepin (5000 U.S.P. units sodium heparin/ml) was given through it into the animal. This catheter was used for the measurement and monitoring of the perfusion pressure in the aorta. A large plastic catheter was inserted into the inferior vena cava, advanced through the right atrium into the right ventricle, and tied in the position, and warm normal saline (37°) was perfused through it to wash the heart free of blood. This catheter was used for the collection of the perfusion fluid from the right ventricle during the experiment.

All branches of the aortic arch were ligated. The descending aorta was cross-clamped and a plastic cannula was inserted into the aortic arch distally to the branch with the catheter and tied in the position. The pulmonary vessels, as well as both superior caval veins, were ligated and cut. The heart with attached cannula and both catheters was removed from the chest, and the aortic cannula was connected with a rubber tube filled with perfusion fluid and coming from a polystaltic pump apparatus. The perfusion fluid was pumped from a reservoir into the aorta and

coronary circulation of the heart and collected by the catheter placed in the right ventricle. The average wet weight of the heart was 5.1 ± 0.1 g, and the perfusion flow rate was kept constant for each heart and represented 7–8 ml/min.

Modified Krebs–Henseleit bicarbonate solution containing, in m-moles/l, NaCl, 118.5; KCl, 4.7; $CaCl_2$, 1.3; $KH_2PO_4$, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 24.9; and D-glucose, 5.5; and equilibrated with a gas mixture of 95% $O_2$ + 5% $CO_2$, was used as a perfusion medium. The $pO_2$ of this medium ranged from 550 to 640 mm Hg, and $pCO_2$ from 17 to 24 mm Hg. The pH and temperature of the medium were constantly monitored and kept stable at 7.4 and 37° respectively.

A tension of 10 g was applied to the right ventricle of each heart. The right ventricular performance was assessed by measuring of a wall tension which was recorded with a Grass force displacement transducer FT 03C and Grass model 5 polygraph, and was expressed in mm of deflecting pen. The left ventricular performance was assessed by means of an isovolumetric rubber balloon introduced into the left ventricle through the left atrial appendage and the mitral valve. The pressure developed within the balloon filled with normal saline was recorded with Statham P23D pressure transducer and Grass polygraph, and was expressed in mm Hg. The first derivative of developed pressure within the balloon, $dP/dt$, was recorded simultaneously.

Each heart was first perfused with the above-mentioned medium for 20 min of an equilibration period, which was immediately followed by 10 min of an experimental period. During this period, hearts were either perfused with the same medium in the control group, or with Krebs bicarbonate medium containing experimental drugs. Hearts were frozen while beating in liquid nitrogen at the end of the experimental period and kept frozen until biochemically analyzed.

Propranolol-treated hearts were perfused with Krebs bicarbonate medium as described above and containing $10^{-5}$ M propranolol hydrochloride from the start of the equilibration period until the end of the experiment.

The outflow medium from the right ventricle was collected in 5-min intervals during the end of the equilibration and throughout the experimental period. All outflow media were collected in ice-cold calibrated vessels, volumes measured and media saved frozen until biochemically analyzed.

The ventricles of frozen hearts were dissected from other tissue, weighed out frozen, and 1 g tissue was immediately homogenized in 4 ml of 5% ice-cold perchloric acid. The ventricles were first homogenized with Sorvall Omni Mixer at high speed for 30 sec at 0°, and then rehomogenized with a Potter–Elvehjem glass homogenizer at 0°. The homogenates were centrifuged at 5000 *g* for 10 min at 4°. The measured parts of supernatants were neutralized with 1 N KOH to pH 7.0, the volumes estimated and the fluids biochemically analyzed. The sediments free of supernatants were dissolved in 40 ml of 1 N NaOH and diluted with distilled water, and protein content was estimated. Myocardial concentration of any substrate was calculated from the concentration of the substrate in supernatant fluid and total volume of supernatant, and was expressed in μmoles or nmoles/g of myocardial protein.

The measured parts of outflow media were similarly neutralized with 1 N KOH to pH 7.0, the volumes estimated, and fluids biochemically analyzed. Glucose uptake by the heart was calculated from the difference of glucose concentrations measured in inflow and outflow media, and the volume of outflow medium. Under the present experimental conditions, the concentration of glucose was decreased from 100 ± 1 to 84 ± 1.5 mg/100 ml in the perfusion fluid after its single circulation through the heart. The total amount of metabolite released from the heart was calculated from its concentration in the outflow medium and the volume of the outflow medium.

These biochemical methods were used: glucose was determined enzymatically by hexokinase [8]; lactate and pyruvate were measured enzymatically with lactate dehydrogenase [9, 10]; glycogen was measured colorimetrically by the anthrone method of Seifter *et al.* [11]; glycerol was determined enzymatically with pyruvate kinase, glycerol kinase and lactate dehydrogenase [12]; free fatty acid (FFA) were measured colorimetrically according to the method of Dalton and Kowalski [13]; adenosine triphosphate (ATP) was determined enzymatically with glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase [14]; adenosine diphosphate (ADP) was determined enzymatically with lactate dehydrogenase and pyruvate kinase [15]; and endogenous inosine was measured enzymatically with xanthine oxidase and nucleoside phosphorylase [16]. Protein content was measured colorimetrically according to the method of Lowry *et al.* [17]. The present values of myocardial contents of ATP, ADP and glycogen, if recalculated per g wet or dry tissue, are similar to those obtained by others [18–20].

*Experiments with rat diaphragm muscles.* Wistar male rats weighing between 120 and 160 g were allowed free access to food and water until used for these experiments. The preparation of diaphragm muscles and their incubation in Krebs bicarbonate medium containing 5.5 m-moles D-glucose have been described before [21]. After incubation, muscles were quickly removed, digested in hot (100°) 30% KOH and analyzed for glycogen according to the method of Seifter *et al.* [11].

Incubation media were biochemically analyzed; glucose concentration was determined enzymatically by the hexokinase method [8]; lactate and pyruvate were determined enzymatically with lactate dehydrogenase [9, 10]. The amount of glucose taken up by the muscle was calculated from its starting and final concentrations in the medium, and the volume of the incubation medium; glucose uptake was expressed in μmoles/g wet tissue. Lactate or pyruvate released by the muscle was calculated from its concentration in the medium and the volumes of the medium; values were expressed in μmoles or nmoles/g wet tissue.

Inosine and uridine, as well as all enzymes for biochemical determinations, were obtained from C. H. Boehringer and Sohne CmbH. Propranolol hydrochloride was obtained from Ayerst Co., and epinephrine hydrochloride from Vitarine Co. All chemicals used in these experiments were purchased from J. T. Baker Chemical Co.

The present data were expressed as a mean ± S.E.M., and were statistically evaluated by Student's *t*-test.

## RESULTS

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on right ventricular wall tension, developed pressure in the left ventricular balloon, dP/dt and heart rate in isolated perfused rabbit hearts.* At the beginning of the experimental period, right ventricular wall tension, developed pressure in the left ventricular balloon and heart rate of control hearts were 16.6 ± 1.2 mm, 45.0 ± 4.4 mm Hg and 102 ± 2 beats/min, respectively, and all these values remained unchanged during the entire experiment. Inosine in a concentration of $10^{-5}$ M increased right ventricular wall tension, developed pressure in the left ventricular balloon and its d*P*/d*t* by 40 per cent ($P < 0.01$), 62 per cent ($P < 0.0125$) and 57 per cent ($P < 0.025$) respectively. Similarly, uridine increased right ventricular wall tension by 30 per cent ($P < 0.01$), developed pressure in the left ventricular balloon by 78 per cent ($P < 0.0125$) and its d*P*/d*t* by 66 per cent ($P < 0.0025$). Both nucleosides were without effect on the heart rate of isolated rabbit hearts.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on myocardial glucose uptake, and the release of lactate, glycerol and FFA from isolated perfused rabbit hearts.* Myocardial glucose uptake of control hearts was 56.9 ± 5.6 μmoles glucose/g of protein during the pre-experimental period of 5 min, and this value remained unchanged during the entire experiment (Table 1). While the nucleosides had no effect on myocardial glucose uptake during the first half, inosine and uridine increased it by 52 per cent ($P < 0.025$) and 66 per cent ($P < 0.01$), respectively, during the second half of the experiment.

Control rabbit hearts released 26.3 ± 2.4 μmoles lactate/g of protein, 1.9 ± 0.1 μmoles glycerol/g of protein and 4.5 ± 0.4 μmoles FFA/g of protein during the pre-experimental period. All these values remained unchanged in control hearts during the entire experiment. Inosine and uridine had no effect on the release of lactate, glycerol and FFA from isolated rabbit hearts.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on myocardial contents of glycogen, lactate, pyruvate, FFA and glycerol in isolated perfused rabbit hearts.* Myocardial content of glycogen was 88.6 ± 5.5 μmoles glucose/g of protein in control hearts at the end of the experiment (Table 2). Myocardial glycogen remained unchanged in inosine, but increased 31 per cent above control value in uridine-perfused hearts.

Myocardial contents of lactate and pyruvate were 28.0 ± 2.7 μmoles and 573 ± 45 nmoles/g of protein, respectively, in control hearts at the end of the experiment. The content of lactate increased by 47 and 83 per cent and pyruvate by 23 and 40 per cent in inosine and uridine perfused hearts respectively.

Myocardial contents of FFA and glycerol were 10.5 ± 1.2 and 7.6 ± 0.5 μmoles/g of protein in control hearts at the end of the experiment. Inosine had no effect on myocardial content of FFA, but increased the amount of glycerol by 32 per cent. Uridine had no effects on the contents of FFA and glycerol in rabbit hearts.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on myocardial contents of ATP, ADP and endogenous inosine in isolated perfused rabbit hearts.* Myocardial contents of ATP, ADP and endogenous inosine were 29.8 ± 2.8, 8.9 ± 0.6 and 1.0 ± 0.2 μmoles/g of protein, respectively, in control hearts at the end of the experimental period (Table 3). While the content of ATP decreased by 30 per cent, the contents of ADP and endogenous inosine increased by 17 and 160 per cent above control values, respectively, in inosine-perfused hearts. Similarly, the myocardial content of ATP decreased by 27 per cent, and the contents of ADP and endogenous inosine increased by 24 and 270 per cent above control values, respectively, in uridine-perfused hearts.

*Effect of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on right ventricular wall tension, developed pressure in the left ventricular balloon, dP/dt and heart rate in isolated propranolol-treated rabbit hearts.* In propranolol-treated hearts, right ventricular wall tension, devel-

Table 1. Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on the uptake of glucose by isolated perfused normal and propranolol-treated rabbit hearts*

| | Uptake of glucose (μmoles/g protein/5 min) | | |
|---|---|---|---|
| | Pre-experimental period (5 min) | Experimental period | |
| | | First half (5 min) | Second half (5 min) |
| Control | 56.9 ± 5.6 (10) | 62.2 ± 3.4 (10) | 50.1 ± 11.5 (10) |
| Inosine, $10^{-5}$ M | 56.8 ± 5.3 (12) | 68.0 ± 7.5 (12) | 86.1 ± 13.9† (12) |
| Uridine, $10^{-5}$ M | 58.6 ± 5.0 (10) | 71.8 ± 7.6 (10) | 97.3 ± 16.7‡ (10) |
| Propranolol control | 52.4 ± 9.8 (9) | 48.4 ± 10 (9) | 47.4 ± 8 (9) |
| Propranolol + inosine, $10^{-5}$ M | 45.6 ± 4.7 (9) | 54.8 ± 6.6 (9) | 38.9 ± 4.3 (9) |
| Propranolol + uridine, $10^{-5}$ M | 51.1 ± 8.6 (8) | 64.0 ± 11 (9) | 100.6 ± 21† (9) |

* Values are means ± S. E. The number of experiments is indicated in parentheses.
† $P < 0.025$.
‡ $P < 0.01$.

Table 2. Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on the contents of glycogen, lactate, pyruvate, FFA and glycerol in isolated perfused normal and propranolol-treated rabbit hearts*

| | Glycogen (μmoles glucose/ g protein) | P < | Lactate (μmoles/g protein) | P < | Pyruvate (nmoles/g protein) | P < | FFA (μmoles/g protein) | P < | Glycerol (μmoles/g protein) | P < |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 88.6 ± 5.5 (9) | | 28.0 ± 2.7 (8) | | 573 ± 45 (9) | | 10.5 ± 1.2 (9) | | 7.6 ± 0.5 (10) | |
| Inosine, $10^{-5}$ M | 91.9 ± 10.1 (10) | NS† | 41.2 ± 4.2 (9) | 0.0125 | 704 ± 45 (10) | 0.05 | 9.2 ± 0.7 (9) | NS | 10.0 ± 0.5 (10) | 0.005 |
| Uridine, $10^{-5}$ M | 115.7 ± 10.2 (9) | 0.025 | 51.2 ± 7.2 (9) | 0.01 | 801 ± 91 (9) | 0.025 | 10.5 ± 1.1 (9) | NS | 8.6 ± 0.6 (9) | NS |
| Propranolol control | 63.6 ± 6.9 (7) | | 32.4 ± 3.0 (7) | | 542 ± 76 (7) | | 5.5 ± 0.6 (7) | | 6.2 ± 1.1 (7) | |
| Propranolol + inosine, $10^{-5}$ M | 70.3 ± 8.9 (9) | NS | 33.0 ± 5.0 (9) | NS | 601 ± 106 (9) | NS | 6.8 ± 0.6 (9) | NS | 6.9 ± 0.8 (9) | NS |
| Propranolol + uridine, $10^{-5}$ M | 94.2 ± 6.9 (8) | 0.005 | 44.3 ± 4.2 (9) | =0.025 | 657 ± 96 (9) | NS | 6.5 ± 0.7 (9) | NS | 7.6 ± 0.9 (9) | NS |

* Values are means ± S. E. The number of experiments is indicated in parentheses.
† Not significant.

oped pressure in the left ventricular balloon and heart rate were 11.3 ± 1.1 mm, 22.6 ± 1.8 mm Hg and 74 ± 8 beats/min, respectively, at the beginning of the experiment. All these values remained unchanged in control and inosine-treated hearts during the entire experiment.

On the other hand, uridine increased right ventricular wall tension by 39 per cent ($P < 0.05$), developed pressure in the left ventricular balloon by 57 per cent ($P < 0.01$) and its $dP/dt$ by 24 per cent ($P < 0.001$). Both nucleosides were without effect on heart rate of propranolol-treated rabbit hearts.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on the uptake of glucose, and the release of lactate, glycerol and FFA from isolated propranolol-treated rabbit hearts.* Myocardial glucose uptake of propranolol-treated rabbit hearts was 52.4 ± 9.8 μmoles/g of protein during the pre-experimental period (Table 1). This value remained unchanged in control and inosine-perfused hearts during the entire experiment. While uridine had no effect on glucose uptake during the first half, this nucleoside increased it by 97 per cent ($P < 0.025$) during the second half of the experiment.

Propranolol-treated rabbit hearts released 20.6 ± 2.0 μmoles lactate, 3.3 ± 0.2 μmoles glycerol and 3.0 ± 0.5 μmoles FFA/g of protein during the pre-experimental period of 5 min. All these values remained unchanged in control, inosine- and uridine-treated hearts during the entire experiment.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on myocardial contents of glycogen, lactate, pyruvate, FFA and glycerol in isolated propranolol-treated rabbit hearts.* Myocardial content of glycogen was 63.6 ± 6.9 μmoles glucose/g of protein in control propranolol-treated hearts at the end of the experiment (Table 2). While inosine was without effect, uridine increased the content of glycogen by 48 per cent. The contents of lactate and pyruvate were 32.4 ± 3.0 μmoles and 542 ± 76 nmoles/g of protein, respectively, in control propranolol-treated hearts. Inosine had no effect, while uridine increased the content of lactate by 37 per cent. Both nucleosides had no effects on the content of pyruvate in propranolol-treated hearts.

Myocardial contents of FFA and glycerol were 5.5 ± 0.6 and 6.2 ± 1.1 μmoles/g protein, respectively, in control propranolol-treated hearts. Inosine and uridine had no effect on myocardial contents of FFA and glycerol in propranolol-treated rabbit hearts.

*Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on myocardial contents of ATP, ADP and endogenous inosine in isolated propranolol-treated rabbit hearts.* Myocardial contents of ATP and ADP were 16.5 ± 1.8 and 8.2 ± 0.4 μmoles/g of protein, respectively, in control propranolol-treated hearts at the end

Table 3. Effects of inosine ($10^{-5}$ M) and uridine ($10^{-5}$ M) on the contents of ATP, ADP and endogenous inosine in isolated perfused normal and propranolol-treated rabbit hearts*

| | ATP (μmoles/g protein) | P < | ADP (μmoles/g protein) | P < | Endogenous inosine (μmoles/g protein) | P < |
|---|---|---|---|---|---|---|
| Control | 29.8 ± 2.8 (7) | | 8.9 ± 0.6 (7) | | 1.0 ± 0.2 (7) | |
| Inosine, $10^{-5}$ M | 20.8 ± 3.2 (7) | 0.025 | 10.4 ± 0.6 (7) | 0.05 | 2.6 ± 0.8 (8) | 0.05 |
| Uridine, $10^{-5}$ M | 21.9 ± 2.2 (7) | 0.025 | 11.0 ± 0.6 (7) | 0.025 | 3.7 ± 0.7 (8) | 0.005 |
| Propranolol control | 16.5 ± 1.8 (7) | | 8.2 ± 0.4 (7) | | 3.2 ± 0.8 (7) | |
| Propranolol + inosine, $10^{-5}$ M | 18.1 ± 1.7 (8) | NS† | 8.0 ± 0.5 (9) | NS | 2.6 ± 0.5 (8) | NS |
| Propranolol + uridine, $10^{-5}$ M | 26.0 ± 2.4 (8) | 0.005 | 9.7 ± 0.6 (9) | 0.05 | 1.7 ± 0.4 (8) | NS |

* Values are mean ± S. E. The number of experiments is indicated in parentheses.
† Not significant.

Table 4. Effects of different concentrations of inosine and uridine on the uptake of glucose, the content of glycogen and the production of lactate and pyruvate by rat diaphragm muscles incubated for 60 min in Krebs medium*

| | Glucose uptake (μmoles/g wet tissue) | P < | Glycogen (μmoles glucose/g wet tissue) | P < | Lactate (μmoles/g wet tissue) | P < | Pyruvate (nmoles/g wet tissue) | P < |
|---|---|---|---|---|---|---|---|---|
| Control | 6.9 ± 0.6 (10) | | 25.9 ± 1.2 (13) | | 28.8 ± 1.1 (13) | | 863 ± 33 (10) | |
| Inosine, $10^{-4}$ M | 9.4 ± 1.1 (10) | 0.05 | 23.4 ± 1.1 (13) | NS† | 35.5 ± 2.0 (13) | 0.005 | 971 ± 55 (10) | NS |
| $10^{-5}$ M | 9.1 ± 0.9 (10) | 0.05 | 23.6 ± 1.1 (13) | NS | 32.1 ± 1.6 (13) | 0.05 | 881 ± 36 (10) | NS |
| $10^{-6}$ M | 9.0 ± 1.0 (10) | 0.05 | 22.7 ± 1.0 (13) | NS | 33.2 ± 1.7 (13) | 0.025 | 883 ± 34 (10) | NS |
| Control | 7.2 ± 0.5 (8) | | 25.5 ± 1.1 (9) | | 38.2 ± 2.6 (10) | | 795 ± 49 (9) | |
| Uridine, $10^{-4}$ M | 10.9 ± 1.8 (8) | 0.05 | 29.7 ± 1.1 (9) | 0.01 | 36.0 ± 2.7 (10) | NS | 896 ± 45 (9) | NS |
| $10^{-5}$ M | 11.3 ± 1.1 (8) | 0.0025 | 30.0 ± 2.0 (9) | 0.05 | 35.7 − 3.6 (10) | NS | 806 ± 60 (9) | NS |
| $10^{-6}$ M | 10.4 ± 0.9 (8) | 0.005 | 31.4 ± 2.3 (9) | 0.025 | 34.7 ± 2.3 (10) | NS | 905 ± 47 (9) | NS |

* Values are means ± S. E. The number of experiments is indicated in parentheses.
† Not significant.

of the experiment (Table 3). While inosine caused no changes, uridine increased the content of ATP by 58 per cent and ADP by 18 per cent above the control values.

The contents of endogenous inosine was 3.2 ± 0.8 μmoles/g of protein in control propranolol-treated hearts. Both nucleosides had no effect on the content of endogenous inosine of propranolol-treated hearts.

*Effects of different concentrations of uridine on the uptake of glucose, content of glycogen, and the production of lactate and pyruvate by rat diaphragm muscles.* The uptake of glucose by control rat diaphragm muscle was 7.2 ± 0.5 μmoles/g wet tissue, and the muscle content of glycogen was 25.5 ± 1.1 μmoles glucose/g wet tissue after 60 min of incubation in Krebs bicarbonate medium containing 5.5 m-moles D-glucose (Table 4). The concentrations of lactate and pyruvate found in the medium after the incubation of control muscles were 38.2 ± 2.6 μmoles/g wet tissue and 795 ± 49 nmoles/g wet tissue respectively.

The uptake of glucose by diaphragm muscle was increased by 51, 57 and 44 per cent above control value in the presence of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M uridine respectively. Uridine in concentrations of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M also increased the content of glycogen in diaphragm muscle by 16, 17 and 23 per cent respectively. The concentrations of lactate and pyruvate found in the medium after the incubation were unchanged from control values in the presence of $10^{-4}$ to $10^{-6}$ M uridine.

When control rat diaphragm muscles were incubated in Krebs bicarbonate buffer without glucose for 60 min, the content of glycogen was 15.7 ± 1.0 μmoles glucose/g wet tissue, and the concentrations of lactate and pyruvate in the medium were 24.9 ± 1.5 μmoles/g wet tissue and 831 ± 38 nmoles/g wet tissue respectively. Uridine in concentrations of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M had no effect on the content of glycogen in diaphragm muscle, as well as on the concentration of lactate in the medium. Uridine in a concentration of $10^{-4}$ M increased the concentration of pyruvate in the medium by 17 per cent while no effect was observed in the presence of $10^{-5}$ and $10^{-6}$ M uridine.

*Effects of different concentrations of inosine on the uptake of glucose, tissue content of glycogen and the production of lactate and pyruvate by rat diaphragm muscle.* The uptake of glucose by control diaphragm muscles was 6.9 ± 0.6 μmoles glucose/g wet tissue and their content of glycogen was 25.9 ± 1.2 μmoles glucose/g wet tissue after 60 min of incubation (Table 4). There were 28.8 ± 1.1 μmoles lactate and 863 ± 33 nmoles pyruvate/g of wet tissue in the medium after the incubation.

The uptake of glucose by the muscle was increased by 36, 32 and 30 per cent above the control value in the presence of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M inosine respectively. Inosine in concentrations of $10^{-4}$ to $10^{-6}$ M had no effect on the content of glycogen in the muscle. The concentration of lactate in the medium was increased by 23, 11 and 15 per cent above control values in the presence of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M inosine respectively. There was no effect of inosine in concentrations of $10^{-4}$ to $10^{-6}$ M on the concentration of pyruvate in the medium.

## DISCUSSION

In the present study, the infusion of inosine or uridine in concentrations of $10^{-5}$ M into the isolated rabbit hearts caused the increase in right ventricular wall tension and in developed pressure in the left ventricular balloon, as well as in $dP/dt$. Since neither nucleoside had an effect on the heart rate of isolated rabbit hearts, it is clear that both inosine and uridine increased myocardial contractility of isolated rabbit hearts.

Uridine, but not inosine, in concentration of $10^{-5}$ M also increased right ventricular wall tension, developed pressure in the left ventricular balloon, as well as $dP/dt$ in isolated propranolol-treated hearts. It is obvious that uridine increased myocardial contractility in propranolol-treated hearts. On the other hand, propranolol, which blocks inotropic and chronotropic responses to catecholamines, inhibited the inotropic effect of inosine in rabbit hearts.

The infusion of inosine or uridine in concentrations of $10^{-5}$ M into isolated rabbit hearts increased the

uptake of glucose by the myocardium, but caused no change in the amount of lactate released from these hearts. On the other hand, both nucleosides increased myocardial contents of lactate and pyruvate in rabbit hearts. While inosine caused no change, uridine increased myocardial content of glycogen. The content of any metabolite in the myocardium is the result of two opposite processes, and the accumulation of this metabolite is caused either by its increased production, an inhibition of its utilization, or by a combination of both. Since the accumulations of lactate and pyruvate occurred simultaneously with enhanced glucose uptake and myocardial contractility in inosine- and uridine-perfused hearts, it seems very likely that both nucleosides increased the production of these metabolites of glycolysis.

Increased muscular contraction itself has been shown to accelerate the membrane transport of a number of hexoses and pentoses [22], and therefore, could explain the increased glucose uptake in inosine- and uridine-perfused hearts. However, in the present study, inosine and uridine in concentrations of $10^{-4}$ to $10^{-6}$ M increased the uptake of glucose in incubated quiscent rat diaphragm muscles where the interfering action of contraction was absent. The production of lactate by the muscle was increased only in the presence of inosine. On the other hand, uridine increased glycogen content of the muscle incubated in the presence of glucose. Since the effect of uridine on the content of glycogen was absent in muscles incubated without glucose in the medium, it seems very likely that this nucleoside stimulated the synthesis of glycogen in rat diaphragm muscles. If we can apply these results observed in skeletal muscle to the myocardium, then both nucleosides stimulated glucose uptake by the myocardium primarily, and not secondary to increased contractility, and uridine accumulated glycogen in rabbit hearts due to its increased synthesis.

Both inosine and uridine failed to change the amounts of FFA and glycerol released from rabbit hearts. Inosine-perfused hearts contained more glycerol, but the content of FFA remained unchanged. Uridine caused no change in myocardial contents of both end products of lipolysis. The accumulation of glycerol in inosine-treated hearts is not clear, but it seems that inosine, as well as uridine, had no effect on myocardial lipolysis. On the other hand, inosine and uridine were observed to inhibit not only the basal, but also the catecholamine-stimulated lipolysis in isolated rat adipose tissue [21].

We observed increased breakdown of high energy phosphate compounds in rabbit hearts perfused with inosine and uridine, since the myocardial content of ATP decreased and the content of ADP was increased. One of the degradative products of AMP, endogenous inosine, was also accumulated in inosine- and uridine-perfused hearts. The increased breakdown of ATP in these hearts was most probably due to higher energetic requirements for increased myocardial contractility.

In the present study, propranolol-treated hearts contained less glycogen and ATP than nontreated rabbit hearts. The cause for this is unclear from the present results, but it is possible that propranolol inhibited the synthesis of ATP and glycogen in the heart. Since propranolol inhibits the activities of glycogenolytic and lipolytic rates through the inhibition of beta-receptor sites [23, 24], a decreased production of metabolites from these metabolic pathways would produce fewer substrates for the oxidative phosphorylation, and inevitably lower synthesis of ATP. It seems that some effects of propranolol are independent of the blockade of beta receptors, because this drug has been shown to inhibit oxidative phosphorylation of heart mitochondria [25], the incorporation of pyruvate into $CO_2$ and lipids of epididymal fat tissue [26] and myocardial glucose uptake [27], as well as to depress the contents of high energy phosphate compounds of isolated rat heart [27].

Increased glucose uptake, glycolysis and the breakdown of ATP caused by inosine in rabbit hearts were inhibited by propranolol. On the other hand, uridine had inotropic and metabolic effects in propranolol-treated hearts similar to the effects in non-treated hearts. Increased myocardial contractility was accompanied by higher glucose uptake, glycogen synthesis and glycolysis in propranolol-treated hearts perfused with uridine. However, higher contents of ATP and ADP caused by uridine in propranolol-treated hearts are not clear. It can be only speculated that this nucleoside enhanced the supply of substrates from the glycolytic pathway for the Krebs cycle and, thereby, increased the synthesis of ATP in propranolol-treated hearts.

While the action of uridine seems to be independent of adrenergic mechanisms, the inhibition of inotropic and metabolic effects of inosine in rabbit hearts by propranolol supports the idea that the action of this nucleoside is mediated by endogenous catecholamines. Then, one would expect that such metabolic pathways as glycogenolysis or lipolysis, which are stimulated by catecholamines, would also be activated by inosine. However, we could not observe any signs of stimulation of these pathways in the heart perfused with inosine. Moreover, this nucleoside stimulated the uptake of glucose, a process which is not believed to be under the control of adenyl cyclase [28], and therefore it seems likely that the metabolic and inotropic effects of inosine are not mediated by endogenous catecholamines. The inhibition of myocardial action of inosine by propranolol is unclear, but might be due to general depressing action of this beta blocker [25–27]. However, further studies will have to be carried out to determine the role of the release of catecholamines in the action of inosine in the heart.

Metabolic effects of inosine and uridine appear to be similar to those described for cardiac glycosides. Ouabain or digoxin stimulated glucose uptake in the heart [18] and glucose uptake and glycogen synthesis in diaphragm muscle [29], as well as inhibited the catecholamine-stimulated lipolysis in adipose tissue [30]. The similarity in actions between nucleosides and cardiac glycosides is even closer, since all these compounds are positive inotropic agents. The fact that inosine and uridine in such low concentrations as $10^{-6}$ or $10^{-7}$ M were capable of changing the metabolism of muscle or adipose tissue [21] suggests that specific receptors are involved in their mechanism of action rather than effects on intracellular contents of nucleotides. Further studies will be

required to determine if inosine or uridine, like cardiac glycosides, inhibits $Na^+$-$K^+$-activated ATPase.

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