

Changes of nucleotide content in human and rat heart during cardiac surgery and ischemia*

Ryszard T. Smoleński, Andrzej C. Składanowski, Julian Świerczyński, Mario Perko^a, Mirosława Narkiewicz^a and Mariusz M. Żydowo**

Department of Biochemistry and ^aDepartment of Cardiac Surgery, Academic Medical School of Gdańsk, Dębinki 1, 80-211 Gdańsk, Poland

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The influence of ischemia on purine nucleotide and their catabolite concentration in human myocardium was investigated during surgery of acquired and congenital heart defects. This was compared with the influence of ischemia on rat heart. Concentrations of adenine and guanine nucleotides and their catabolites were measured in the extracts of heart biopsies taken at the onset of ischemia and at the time of reperfusion. The content of myocardial ATP in human heart decreased from the initial value of 22.3 ± 1.1 to 14.6 ± 1.5 nmol/mg protein and total adenine nucleotide pool decreased from 34.2 ± 1.8 to 27.6 ± 1.5 nmol/mg protein during the operation. Significant increases in myocardial concentrations of purine catabolites were also observed with the most prominent rise in inosine from below 0.5 at the onset of the ischemia to 3.0 ± 0.5 nmol/mg protein at the time of reperfusion. A positive correlation was demonstrated between the concentration of purine catabolites in the heart at the end of ischemia with the decrease of both ATP and the total nucleotide pool. An interesting metabolic specificity of the ischemic human heart appeared to be only a small accumulation of inosine monophosphate (IMP). The increase of IMP in the rat heart after ischemia was several-fold higher.

Thus, cardiac surgery of congenital and acquired heart defects was associated with a significant decrease in myocardial adenylate pool and a single biopsy collected at the end of ischemia seems to be sufficient to evaluate the extent of this metabolic and possibly functional impairment of the heart.

Metabolism of adenine nucleotides in the heart is directly linked to the high energy phosphate turnover and to the mechanical work of the heart [1]. Adenine nucleotide metabolites play also an important role, through adenosine and cyclic nucleotides, in the metabolic control of many physiological processes including coronary flow, platelet aggregation, inflammatory response and hormone action on the heart

[2 - 6]. According to the recent data, nucleotide pool depletion plays a minor role in functional impairment of the postischemic heart [7] but the influence of the nucleotide pool decrease on functioning of the key feedback control mechanisms of the heart has not been fully explored and there is some evidence that, under these conditions, heart functioning may be severely impaired [8]. Clinical data on the degree of

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**Correspondence to: Professor Mariusz M. Żydowo, Department of Biochemistry, Academic Medical School of Gdańsk, Dębinki 1, 80-211 Gdańsk, Poland

nucleotide pool depletion are conflicting [9 - 13] which is due to a substantial extent by variety of techniques used for cardioprotection. Therefore, further evaluation of this problem is still necessary.

The present study was undertaken to estimate the degree of adenine nucleotide pool depletion in the human heart during operations of congenital and acquired heart defects. The relation between purine catabolite accumulation and nucleotide pool depletion was also evaluated, and the profile of purine catabolites was compared with that in rat heart subjected to ischemia.

METHODS

Collection of human heart biopsy specimens.

This study was carried out during surgery of acquired and congenital heart defects performed at the Department of Cardiac Surgery of the Academic Medical School of Gdańsk. The experimental protocol was approved by the local Ethical Committee. The routine procedure of myocardial preservation consisted of topical cooling if the expected ischemic time was short. If ischemia was longer, the modified Roe's cold cardioplegic solution of the following composition: 27 mM NaCl, 20 mM KCl, 3 mM MgCl₂, 278 mM glucose, 1 mM procaine and 10 mM NaHCO₃ (pH 7.8) was infused. The volume of the administered cardioplegic solution was 12 ml/kg body weight at the first infusion and averaged 6 ml/kg body weight during the second and the third infusion. Transmural biopsy specimens were collected from the apex of the heart using "Tru-Cut" biopsy needles (Travenol Laboratories, Deerfield, IL, U.S.A.) during the course of the operation: the first immediately after clamping of the aorta and the second immediately after de-clamping of the aorta. The blood was removed with gauze and the sample was frozen in liquid nitrogen within 15 s from the time of collection. Biopsy specimens (3 - 10 mg) were extracted with 0.3 ml of 0.6 M perchloric acid in 1.5 ml Eppendorf tubes fitted with pestles as described by Hearse [14]. Extracts were then centrifuged (11000 × g for 5 min) in a cooled micro-fuge before removal and neutralisation of 0.25 ml of the supernatant with approximately 0.09 ml of 2 M potassium hydroxide. Aliquots of

this extract were injected into the chromatograph. Perchloric acid pellets were solubilized with 0.05 M NaOH and protein was determined according to the procedure of Lowry *et al.* [15].

Experimental cardioplegic arrest of the rat heart. Hearts of "Wistar" rats were arrested *in situ* by infusion of 2.5 ml of cold crystalloid cardioplegic solution of similar composition as used in humans. Hearts were then connected to the perfusion apparatus and were kept at 25°C. Infusion of 1.25 ml cardioplegic fluid was then repeated every 30 min at the speed of 1.25 ml/min. After 1 h of cardioplegic arrest, hearts were frozen with aluminium blocks precooled in liquid nitrogen, and extracted in the same way as human heart samples. For determination of nucleotide content at the onset of ischemia rat hearts were frozen immediately after the first infusion of the cardioplegic solution.

Chromatographic procedure. Chromatographic analysis of the extracts was performed using the modified procedure described previously [16]. The chromatographic system consisted of Liquochrom 2010 (Labor MIM, Hungary) apparatus equipped with prepacked reversed phase column type ODS (0.4 × 25 cm, particle size 5 µm, POCh, Lublin, Poland). UV detector was set at 254 nm. The mobile phase consisted of: solution A - 150 mM KH₂PO₄, 150 mM KCl (pH 6.0), and solution B - 15% acetonitrile in solution A. The initial conditions were 100% of solution A running up to 5 min after injection, then a linear gradient was started with solution B increasing up to 100% in 7 min. Solution B was running at 100% for the next 3 min and the column was reequilibrated with solution A for 15 min before the next injection. The low pressure gradient mixer and data acquisition and processing were controlled by the Karat-2 microcomputer (Elkor, Gdańsk, Poland). Suppliers of chemicals for HPLC were described previously [16].

Statistical analysis. Values are reported as the mean (± S.E.M.). Statistical significance of the differences was evaluated using a paired Student *t*-test (comparison of paired human heart biopsies) or unpaired Student *t*-test (comparison of human and rat heart). Regression analyses were based on single data points from Table 1; *P* < 0.05 was considered a significant difference.

RESULTS

Chromatographic evaluation of purine metabolites in the human heart biopsies

Figure 1A presents the chromatogram of the standards of purine metabolites separated on reversed-phase column. Analysis of the human and rat heart extracts separated under the same conditions is presented in Figs. 1 B - E. A decrease in the ATP concentration accompanied by an increase in AMP and purine nucleosides and bases occurred both in human and rat heart after ischemia. However, in the rat heart the peak of IMP was much higher.

Adenine nucleotides and catabolites in the human and rat myocardial tissue during ischemia

The ischemia during cardiac surgery caused substantial decreases in myocardial ATP and in the total adenine nucleotide pool (TAN, Table 1). An increase in inosine, adenosine and hypoxanthine concentration was also demonstrated as the result of ischemia. Notably, there was a very small increase in IMP concentration which accounted for only 4.7% of all catabolites. Rat heart subjected to ischemia after cardioplegic arrest causing a similar depletion of ATP and total adenylate pool showed also an accumulation of nucleosides and bases (Table 2), but the increase in IMP concentration was several fold higher and accounted for 12% of all adenylate catabolites. The concentration of adenosine after ischemia was also significantly greater in the rat heart.

Correlation of purine catabolite accumulation with the depletion of ATP, total adenine nucleotides and adenylate energy charge

To test whether the accumulation of purine catabolites in the biopsies of human heart collected after ischemia would express the extent of metabolic alterations of the adenine nucleotide pool, a series of correlation tests were performed. The sum of adenosine + inosine + hypoxanthine correlated positively with the degree of nucleotide pool depletion and with the decrease in ATP concentration during ischemia (Figs. 2, 3). The concentrations of tissue purine catabolites correlated inversely with post-

ischemic adenylate energy charge (Fig. 4). All these correlations were highly significant.

DISCUSSION

In the present report it has been shown that substantial depletion of adenine nucleotide pool occurred in human heart during cardiac surgery associated with myocardial ischemia. This is not surprising because of the rapid turnover of high energy phosphates and of the almost total oxygen dependence of ATP generation in the heart. However, the available data concerning changes in the adenine nucleotide pool are conflicting and in addition to the results showing small metabolic changes in the heart subjected to cardioplegic arrest and ischemia [12, 13], there is some evidence that the nucleotide pool depletion may be substantial, especially if the heart is not infused with a cardioplegic solution or if it is hypertrophied [9 - 11].

In some studies on animals a correlation between postischemic ATP concentration and mechanical performance of the heart has been reported [1], but this correlation was questioned [7, 17]. On the other hand, it was found that the release of adenosine from the postischemic heart in response to ischemia was markedly reduced [8], which could result in impairment of the adenosine-dependent vasodilatory response and could enhance platelet aggregation and leukocyte infiltration [2 - 6].

An interesting observation is a very small accumulation of IMP in the human heart subjected to ischemia. This contrasts with the observations on the rat or guinea pig heart in which IMP accounts for a substantial part of adenylate catabolic products [18 - 20], and is similar to the situation in the dog heart, in which the accumulation of IMP is very low [21]. These differences in adenylate catabolic profile in hearts of various mammals seem to be a consequence of different species-dependent distribution of enzyme activities. For instance, the activity of AMP-deaminase has been found to be five times greater in rat heart than in human heart [19] and myocardial activity of nucleoside phosphorylases is also different in man and rat [22].

A practical question is what kind of pharmacological intervention could be undertaken to protect the heart against observed metabolic

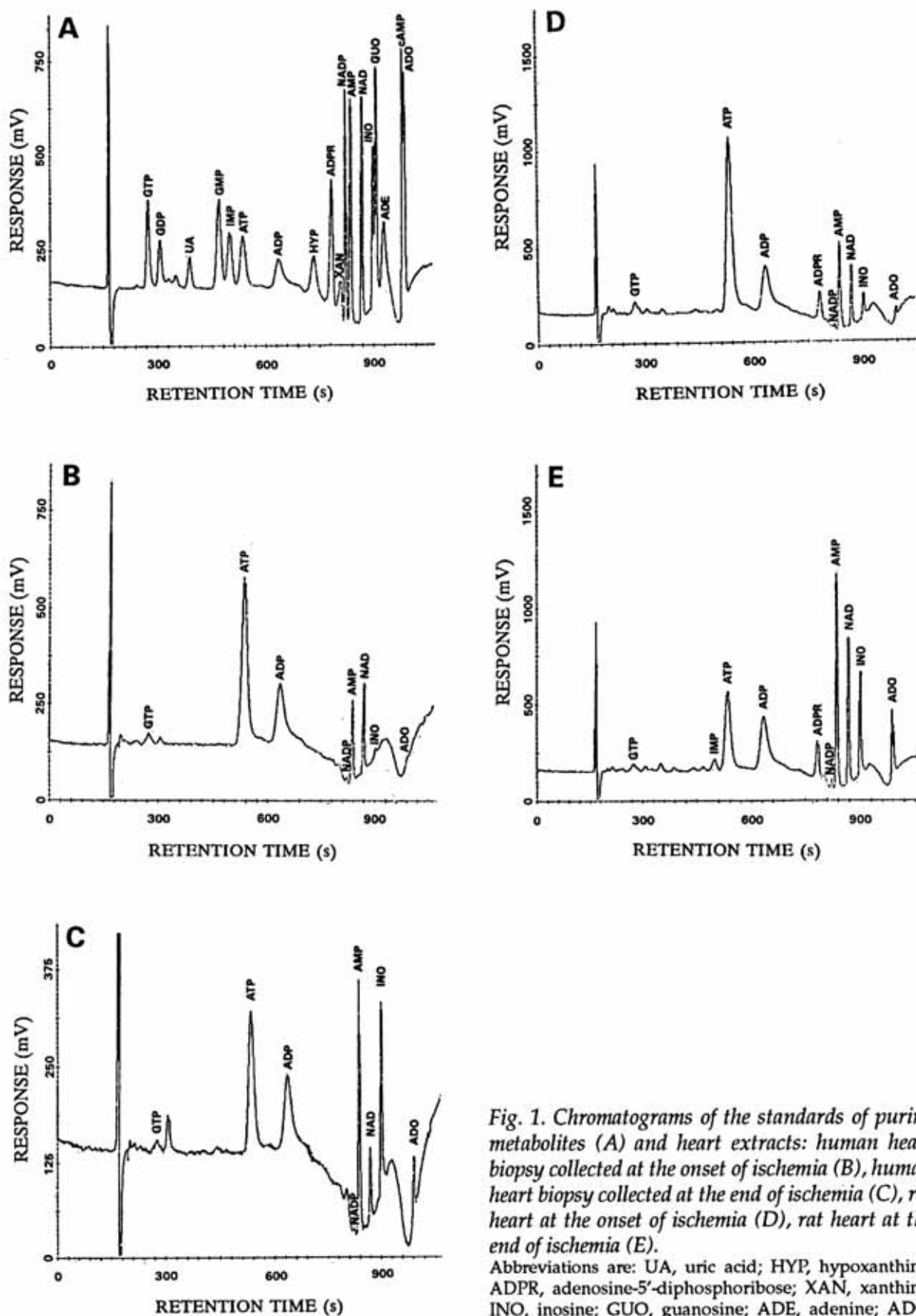


Fig. 1. Chromatograms of the standards of purine metabolites (A) and heart extracts: human heart biopsy collected at the onset of ischemia (B), human heart biopsy collected at the end of ischemia (C), rat heart at the onset of ischemia (D), rat heart at the end of ischemia (E).

Abbreviations are: UA, uric acid; HYP, hypoxanthine; ADPR, adenosine-5'-diphosphoribose; XAN, xanthine; INO, inosine; GUO, guanosine; ADE, adenine; ADO, adenosine.

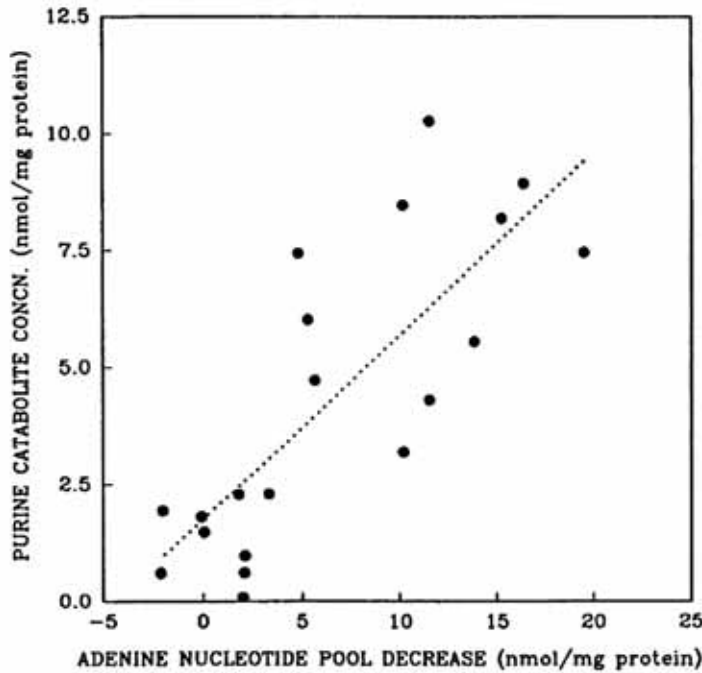


Fig. 2. Correlation between total concentration of purine catabolites (sum of adenosine, inosine and hypoxanthine) in the human heart after ischemia and the depletion of adenine nucleotide pool during ischemia.

The linear regression equation was $y = 0.38x + 1.83$, $r = 0.760$, $P < 0.01$.

changes. The results presented here support the suggestion that it could be beneficial to supply nucleotide precursors: adenosine, inosine or adenine with ribose before or after ischemia [5, 23 - 26]. Alternatively, nucleoside transport blockers [27] or inhibitors of nucleotide degradation [28] could be applied to preserve the endogenous nucleotide pool.

In conclusion, cardiac surgery of congenital and acquired heart defects was found to be associated with a significant decrease of

myocardial adenine nucleotide pool. The estimation of purine catabolite concentration in a single biopsy collected at the end of ischemia may provide information on the extent of this metabolic, and possibly functional impairment of the heart. The species-dependent differences in the profile of the products of the adenine nucleotide degradation indicate that rat heart is not a good model for investigating metabolic changes occurring in human heart during ischemia.

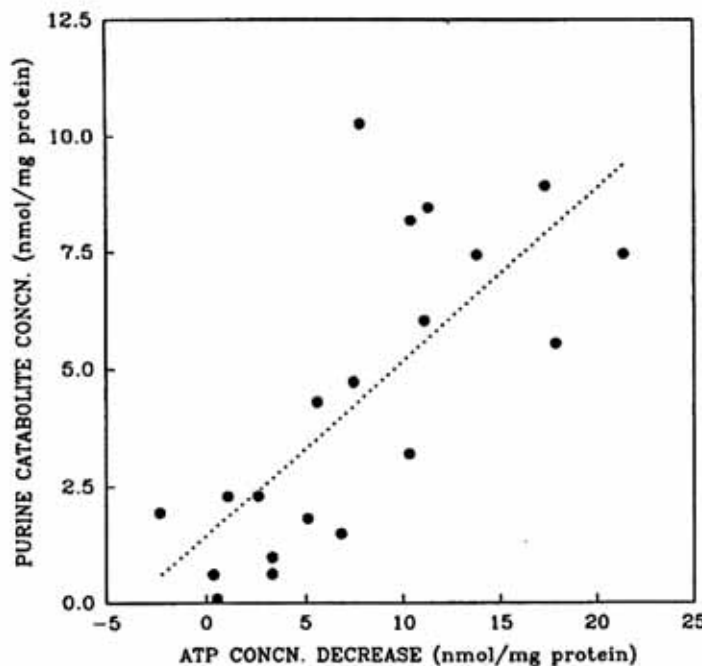


Fig. 3. Correlation between total concentration of purine catabolites (sum of adenosine, inosine and hypoxanthine) in the human heart after ischemia and the decrease in ATP concentration.

The linear regression equation was $y = 0.38x + 1.42$, $r = 0.750$, $P < 0.01$.

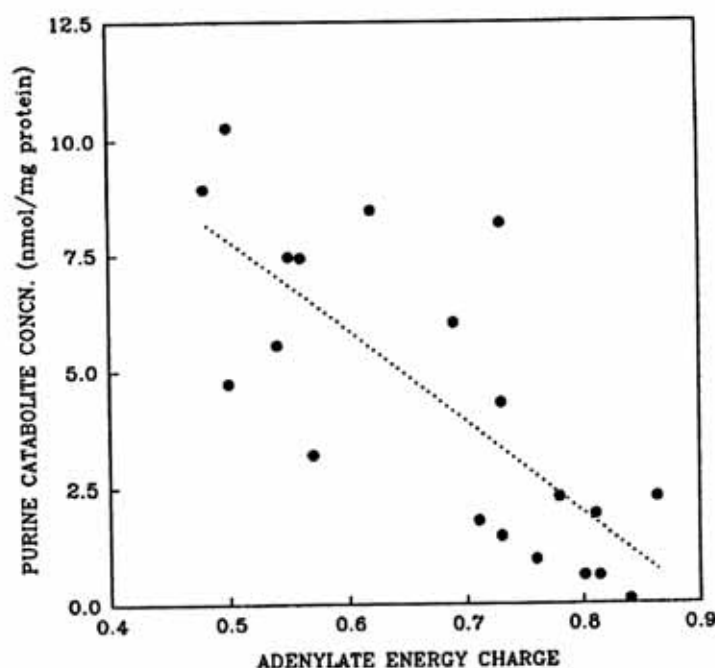


Fig. 4. Correlation between total concentration of purine catabolites (sum of adenosine, inosine and hypoxanthine) in the human heart after ischemia with the adenylate energy charge value.

Linear regression equation was $y = -19.4x + 17.5$, $r = -0.761$, $P < 0.01$.

Table 1

Purine metabolite concentration in the human heart during surgery of congenital and acquired heart defects ($n = 20$)

Purine metabolites at the onset of ischemia												
	ATP	ADP	AMP	TAN	AEC	GTP	IMP	NAD	NADP	ADO	INO	HYP
Average	22.33	9.32	2.44	34.19	0.792	0.922	0.100	2.94	0.181	0.139	0.379	0.306
S.E.M.	1.09	0.81	0.35	1.79	0.016	0.071	0.016	0.22	0.025	0.029	0.109	0.062
Purine metabolites at the time of reperfusion												
Average	14.56 ^a	9.13	3.93 ^b	27.63 ^a	0.679 ^a	0.576 ^a	0.213 ^c	2.81	0.200	0.782 ^b	2.998 ^a	0.553 ^c
S.E.M.	1.51	0.51	0.48	1.49	0.028	0.047	0.045	0.17	0.022	0.184	0.522	0.104

^a $P < 0.001$, ^b $P < 0.01$, ^c $P < 0.02$, comparison with the concentration at the onset of ischemia.

Abbreviations: TAN, total adenine nucleotides; AEC, adenylate energy charge; ADO, adenosine; INO, inosine; HYP, hypoxanthine.

Table 2

Purine metabolite concentration in the rat heart during experimental ischemia after cardioplegic arrest

Purine metabolites at the onset of ischemia ($n = 3$)												
	ATP	ADP	AMP	TAN	AEC	GTP	IMP	NAD	NADP	ADO	INO	HYP
Average	24.70	13.03	3.07	40.80	0.767	0.860	0.090	4.50 ^a	0.120	0.503 ^b	0.280	0.230
S.E.M.	3.01	1.64	0.94	3.62	0.023	0.146	0.047	0.57	0.017	0.170	0.170	0.053
Purine metabolites after 60 min of ischemia ($n = 3$)												
Average	14.34	7.73	6.41	28.48	0.627	0.390	1.184 ^c	4.35 ^d	0.140	3.535 ^c	4.190	0.680
S.E.M.	3.44	0.84	0.67	3.11	0.040	0.129	0.234	0.43	0.010	0.199	1.150	0.032

^a $P < 0.02$, ^b $P < 0.001$, comparison with the concentration at the onset of ischemia in the human heart.

^c $P < 0.001$, ^d $P < 0.005$, comparison with the concentration at the end of ischemia in the human heart.

Abbreviations as in Table 1.

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