Influence of ageing on functional recovery and guanine nucleotide levels of the heart following cold cardioplegic arrest

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Abstract

Objective: The effect of age on metabolism and mechanical recovery of the heart after cardioplegic arrest is important, but remains a relatively unexplored subject. In this study, functional recovery and nucleotide levels were compared in the heart at different ages subjected to prolonged hypothermic cardioplegic arrest. Methods: Three different age groups of rats: 1 (A); 4 (B); and 16 months (C) were perfused in working mode and subjected to cardioplegic arrest (St. Thomas’ No. 1) and ischemia for 4 h at 4°C, followed by reperfusion for 35 min. Cardiac function (cardiac output and aortic pressure) was recorded before and after ischemia. Another series of hearts in all three age groups underwent 5 min of normoxic perfusion to obtain pre-ischemic baseline metabolite concentrations. Hearts were freeze-clamped at the end of each experiment and used for determination of nucleotide and creatine metabolites by HPLC. Results: The post-ischemic recovery (% of the pre-ischemic value) of the cardiac power was 48.9 ± 7.8% for group A, which was significantly higher than the functional recovery of group B (24.1 ± 3.5%) or C (21.4 ± 4.7%, P < 0.05, respectively). There was no difference in ATP or the total adenine nucleotide or creatine metabolite concentrations between the three age groups. In contrast, both GTP and the total guanine nucleotide concentration was highest in A (P < 0.05). Total guanylate pool was 1.52 ± 0.10 1 μmol/g dry wt. in A, as compared to B (1.05 ± 0.04) or C (1.12 ± 0.04). NAD was significantly higher in B (4.1 ± 0.1, P < 0.05), when compared to A (3.6 ± 0.1) and C (3.8 ± 0.1). Conclusion: Best post-ischemic functional recovery after cardioplegic arrest was observed in the 1-month-old hearts (A) and was associated with highest guanine nucleotide concentration; preservation of guanine nucleotide pool in the youngest hearts may be an important mechanism for improved cardioprotection due to the important role of GTP in signalling pathways. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Aging; Ischemia; Cardioplegia; Nucleotide metabolism

1. Introduction

A number of metabolic processes in the heart, i.e. calcium handling, ATPase activity, catecholamine responsiveness and nucleotide metabolism, are known to be modified during ageing [1–3]. Experimental studies in animals suggests that the infant heart is more resistant to ischemia. This has largely been attributed to improved post-ischemic endothelial function [3], reduced calcium handling dysfunction [4,5], decreased 5’-nucleotidase activity [6,7] and increased glycolytic flux [8]. In addition, the senescent heart undergoes a modest degree of myocardial hypertrophy which reduces its tolerance to ischemia [9]. However, clinical experience seems to suggest the opposite relationship between sensitivity to ischemia and age. Both clinical experience [10] and laboratory investigation [1] indicate insufficient protection of immature hearts.
Nucleotides participate in all aspects of cellular metabolism. In addition to the crucial role of ATP in energy metabolism, nucleotides serve as precursors of nucleic acids, are constituents of coenzymes and are involved in regulation. The role of guanine nucleotides in regulation is especially important, since GTP is essential for hormone signalling via G-protein transduction and is also a substrate for cGMP synthesis [11].

Although several studies have evaluated the effects of age related changes in nucleotide metabolism on the recovery of the heart after normothermic ischemia, little is known about the specific changes and their relationship with cardiac function after cardioplegic arrest and hypothermic ischemia, mimicking preservation for cardiac transplantation. In this study, the association between the concentration of ATP, GTP, NAD, NADP and phosphocreatine with functional recovery of the rat heart in a defined age population, after cardioplegic arrest and hypothermic ischemia, was evaluated.

The three age groups used in this rat study were 1, 4 and 16 months; which correspond approximately to the infant/toddler, young adult and middle-aged years of the human species.

2. Methods

2.1. Animals

In all studies, the animals received humane care in compliance with the ‘Principles of Laboratory Animals Care’, formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’, prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1985). Male Sprague-Dawley rats were used in all experiments. Six hearts were studied in each group. Animals were divided into three age groups: (A) 1 month; (B) 4 months and (C) 16 months.

2.2. Experimental preparation and protocol

The animals were anaesthetised with diethyl ether. The femoral vein was exposed and heparin (200 IU) was injected. Exactly 1 min later, the heart was excised and immediately placed in cold (4°C) Krebs Buffer. Approximately 30 s later, the aorta was cannulated and perfusion was started. The isolated working rat heart preparation, which has been described in detail elsewhere [12], was used in this study. Oxygenated Krebs-Henseleit buffer solution was (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 24 mM NaHCO3, 11 mM glucose, 1.2 mM CaCl2 pH 7.4) continuously gassed with a 95% O2–5% CO2 mixture at 37°C from a reservoir 100 cm above the heart and used for perfusion. The hearts were not paced throughout the protocol. Using this preparation, which is essentially that described by Langendorff, the heart will continue to beat, but does not perform external work. After initial Langendorff perfusion for 15 min, conditions were switched into working mode for 20 min. Left atrial pressure was maintained at 15 cm H2O. Perfusion buffer was then spontaneously ejected via an aortic cannula against a hydrostatic pressure of 100 cm H2O. At the end of this phase pre-ischemic coronary flow (CF), cardiac output (CO, sum of aortic and coronary flow) and aortic pressure (AP) were assessed. These indices were used to calculate cardiac power which was calculated according to the formula [13]:

\[ \text{Power (mJ/s per g)} = (P_{\text{dev}} \times CO \times 0.0022)/M. \]

Where power is measured in mJ/s per g, \( P_{\text{dev}} \) is the developed pressure in mmHg (systolic aortic pressure-left atrial pressure), CO is the cardiac output (ml/min) and M is the mass of heart in g.

After taking the readings, hearts were arrested by infusion of 10 ml of St Thomas’ Hospital No. 1 (St1) cardioplegic fluid. St1 supplied as a concentrate (David Bull Labs, Victoria, Australia) was diluted (1:50) in Ringer’s solution (Travenol Laboratories, Norfolk, UK) and filtered before use. After infusion of cardioplegic solution hearts were maintained under hypothermic conditions (4°C) for 4 h. Hearts were then reperfused for 15 min in Langendorff mode followed by perfusion in working mode for the next 20 min. At the end of this phase the post-ischemic function (CF, CO and AP) was evaluated. This experimental protocol is shown in Fig. 1. Hearts were freez-clamped at the end of the experiment. Another series of hearts in all three age groups (n=5–7 in each group) were freeze-clamped after 5 min of normoxic perfusion to obtain pre-ischemic baseline metabolite concentrations.

2.3. Extraction of hearts for metabolic analysis

Tissue extracts were prepared after freeze-drying the hearts overnight. A 40 mg portion of freeze-dried left ventricle of the heart was extracted with 0.4 M perchloric acid at 25 μL/mg dry tissue ratio. The extracts were then centrifuged (13000 × g for 3 min at 4°C) and the supernatant was neutralised with 2 M KOH. After removal of precipitated potassium perchlorate by centrifugation (13000 × g for 3 min at 4°C) extracts were immediately analysed by HPLC.

2.4. Analytical procedures

Nucleotide, nucleoside and base concentrations, as well as creatine metabolites were analysed using reversed-phase [14] or anion-exchange [15,16] HPLC methods described in detail previously. The equipment used was a Merck-Hitachi chromatograph. The re-
versed-phase method was used for determination of ATP, ADP, AMP, NAD, NADP. An anion exchange procedure was used for determination of phosphocreatine, creatine and GTP in heart extracts.

2.5. Statistics

Values are presented as means ± standard error (S.E.M.). Statistical comparison between different age group was performed using one way analysis of variance (ANOVA) followed by Student-Newmann-Keuls test. A value of \( P < 0.05 \) was considered as a significant difference.

3. Results

3.1. Pre-ischemic metabolite concentrations

In the youngest hearts (group A) there was a significantly higher baseline GTP content, which was 1.3 times greater than that of the adult or middle aged hearts of groups B and C (values shown in Table 1). This change was also observed in the concentration of total guanine nucleotides, for the three age groups as shown in Fig. 2. Pre-ischemic concentrations of the other metabolites are displayed in Table 1. Interestingly, the total creatine content of the youngest hearts was significantly lower compared to those of the older age groups; the values are detailed in Table 1.

3.2. Post-ischemic functional and metabolic recovery

The pre-ischemic values of cardiac power for groups A, B and C were 16.48 ± 2.43, 9.37 ± 1.09 and 6.80 ± 0.68 mJ/s per g, respectively. The post-ischemic values were 8.35 ± 1.96, 2.22 ± 0.37 and 1.58 ± 0.40 mJ/s per g for groups A, B and C, respectively. As may be seen in Fig. 3 the post-ischemic recovery (% of the pre-ischemic level) of cardiac power was two times greater in group A than that of B and C. Coronary flow was 9.8 ± 0.6, 13.0 ± 0.9 and 16.4 ± 1.0 ml/min in group A, B and C, respectively, and no significant change was observed after reperfusion with the respective values of 10.5 ± 0.9, 13.3 ± 1.2 and 17.6 ± 2.4 ml/min.

GTP concentration of group A was 1.5 times greater than that of groups B and C as shown in Table 2. This was also reflected in the total guanine nucleotide concentrations of group A, when compared to groups B and C as presented in Fig. 4. NAD content (sum of NAD and ADPR, adenosine diphosphoribose) was highest in group B, as compared to A and C shown in Table 2. This result does not correlate with improved post-ischemic mechanical recovery and guanine nucleotide concentration observed in the infant hearts of group A. Concentrations of all metabolites measured displayed no other significant changes between the groups A, B and C and are shown in Table 2. Unlike pre-ischemic concentration, creatine pool was not significantly different among the groups.

4. Discussion

The present study has shown that hearts from young rats displayed a better recovery of cardiac mechanical function than adult or middle aged animals after prolonged hypothermic cardioplegic arrest. We have demonstrated that this favourable recovery was associated with high GTP content in the youngest group while ATP concentration was similar in different age groups.

Controversy exists concerning the age-related changes in myocardial sensitivity to ischemia. One study which investigated the effect of global ischemia on haemodynamic and metabolic parameters of the rabbit heart failed to find any age-related differences [17]. Other animal studies have shown that young rat hearts are less susceptible to ischemia-reperfusion injury [3,18].

However, all these findings could be species specific and may not apply to the human myocardium. A previous study demonstrated that in the human juvenile heart there is an increase in the release of purines and
Table 1
Metabolite concentrations of non-ischemic hearts at three different age groups

<table>
<thead>
<tr>
<th>Metabolite (μmol/g dry wt)</th>
<th>Group A (1 months)</th>
<th>Group B (4 months)</th>
<th>Group C (16 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>31.9 ± 3.5</td>
<td>31.2 ± 4.3</td>
<td>38.8 ± 2.9</td>
</tr>
<tr>
<td>CREATINE</td>
<td>24.2 ± 1.7</td>
<td>33.2 ± 2.1*</td>
<td>28.9 ± 1.7*</td>
</tr>
<tr>
<td>TCR</td>
<td>56.1 ± 2.1</td>
<td>64.4 ± 2.5*</td>
<td>67.6 ± 1.7*</td>
</tr>
<tr>
<td>ATP</td>
<td>24.1 ± 0.4</td>
<td>24.6 ± 0.9</td>
<td>24.5 ± 0.4</td>
</tr>
<tr>
<td>ADP</td>
<td>4.37 ± 0.13</td>
<td>5.17 ± 0.68</td>
<td>4.37 ± 0.22</td>
</tr>
<tr>
<td>AMP</td>
<td>0.34 ± 0.04</td>
<td>0.63 ± 0.21</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>TAN</td>
<td>28.8 ± 0.4</td>
<td>30.4 ± 0.4</td>
<td>29.2 ± 0.6</td>
</tr>
<tr>
<td>GTP</td>
<td>1.57 ± 0.04</td>
<td>1.24 ± 0.04*</td>
<td>1.27 ± 0.02*</td>
</tr>
<tr>
<td>GDP</td>
<td>0.39 ± 0.01</td>
<td>0.31 ± 0.03*</td>
<td>0.36 ± 0.01*</td>
</tr>
<tr>
<td>GMP</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>NAD</td>
<td>3.88 ± 0.08</td>
<td>3.88 ± 0.07</td>
<td>4.09 ± 0.04</td>
</tr>
<tr>
<td>ADPR</td>
<td>0.17 ± 0.02</td>
<td>0.21 ± 0.06</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>TNAD</td>
<td>4.05 ± 0.07</td>
<td>4.09 ± 0.03</td>
<td>4.22 ± 0.05</td>
</tr>
<tr>
<td>NADP</td>
<td>0.38 ± 0.02</td>
<td>0.43 ± 0.03*</td>
<td>0.48 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means (±S.E.M., n = 7 in groups A and C, n = 5 in group B); *P < 0.05 vs. A.

lactate compared to the adult myocardium during cardiac surgery [1]. This suggests that the younger hearts suffers a greater degree of metabolic injury during cardioplegic arrest [19]. Furthermore, clinical experience suggests that cardioprotection in the infant/child hearts are inadequate during heart surgery [10,20]. However, this apparent discrepancy could be also explained by wide variations in myocardial protection techniques and may include factors such as greater variation in myocardial temperature. It is also known that surgical techniques used to correct congenital defects are more invasive than those used in routine surgery of the adult myocardium. A considerable proportion of both metabolic and functional differences may thus result from mechanical damage.

Comparison of clinical and experimental studies is further complicated by the lack of definition regarding age population within the juvenile heart group. Further investigation is required to determine if the observed changes in nucleotide metabolism of the ischemic rat heart are similar for the human myocardium. However, basic mechanisms of the relationship between nucleotide metabolism and cardiac function are unlikely to be markedly different in the heart of different species. Increased guanine nucleotide levels may be important for improved functional recovery not only in the hearts at different ages but may be important basic mechanism for improved cardioprotection.

The comparison of energy metabolism following ischemia at different ages and evaluation of its significance can be difficult because differences demonstrated are complex, involving alteration of the mitochondrial function, transmembrane action potential and sarcoplasmic reticulum [21]. Changes in nucleotide levels after hypothermic ischemia could be thus secondary

Fig. 2. Total guanine concentration of non-ischemic hearts in different age groups. Values are means (±S.E.M., n = 7 in groups A and C, n = 5 in group B). *P < 0.05 vs. 1 month.

Fig. 3. Recovery of cardiac power following hypothermic (4°C) ischemia for 4 h and reperfusion for 35 min at different age groups. Values are means (±S.E.M., n = 6 in each group). *P < 0.05 vs. 1 month.
to other metabolic alterations. However, the significant role of GTP in the impaired functional recovery cannot be excluded, as GTP was associated with better mechanical recovery of the youngest hearts in this present study.

GTP is essential for normal operation of the G-proteins linked adenylate cyclase system and reduced concentration of GTP may impair regulatory mechanisms in the heart [22]. On the other hand, down-regulation of receptors, such as $\beta$-adrenoceptors which use GTP binding-proteins for their signal transduction mechanisms may be responsible for the observed decline in GTP concentration with age. A reduction in the expression of Gi-α, the functional sub-unit of the G-protein is known to occur in the ageing cardiovascular system [23]. Reduced activity and impaired binding affinity of GTP-binding proteins are another possible explanation [24]. Nitric oxide is an important vasodilator which elicits its actions via stimulation of guanylate cyclase leading to increased levels of cGMP [25]. GTP is the substrate of cGMP. Recent studies have shown a reduction in the release of nitric oxide with age [3,18] decrease in GTP levels observed in the older hearts could be thus responsible for this phenomenon.

Regulation of guanine nucleotide pool involve mainly GMP specific 5'-nucleotidase as catabolic enzyme and IMP dehydrogenase in the synthetic pathway. Regulation of these processes may be age dependent. 5'-Nucleotidase expression is known to change markedly with age. The observed changes could be the result of variations in enzyme levels, coenzyme cycles and protein phosphorylation [26].

The lack of any variation in ATP content, suggests that biochemical pathways which synthesise and utilise this important nucleotide are maintained under the severe conditions of ischemia and cardioplegic arrest, regardless of age dependent differences. Altered NAD content in hearts at different ages may result from differences in NAD breakdown enzymes activities and membrane permeability for NAD during ischemia. There were pre-ischemic differences in NADP content which disappeared after reperfusion. Since we did not measure NADPH, this difference may indicate altered NADP/NADPH ratio or changes in NADP pool. The role of these changes for functional recovery merits further investigation.

In conclusion, the juvenile heart appears to be less susceptible to ischemia-reperfusion injury after cardioplegic arrest and prolonged hypothermic preservation. This was associated with increased guanine nucleotide level. This change may have an important role for myocardial cell metabolism and receptor mediated responses of the adult and infant heart, which could lead to novel therapeutic approaches.

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**Table 2**

Metabolite concentrations of the heart following hypothermic (4°C) ischemia for 4 h and reperfusion for 35 min at different age groups

<table>
<thead>
<tr>
<th>Metabolite (µmol/g dry wt.)</th>
<th>Group A (1 months)</th>
<th>Group B (4 months)</th>
<th>Group C (16 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMP</td>
<td>0.11 ± 0.05</td>
<td>0.06 ± 0.023</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>GDP</td>
<td>0.15 ± 0.06*</td>
<td>0.21 ± 0.03</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>NAD</td>
<td>3.29 ± 0.16</td>
<td>3.63 ± 0.24*</td>
<td>3.35 ± 0.15</td>
</tr>
<tr>
<td>TAN</td>
<td>3.59 ± 0.11</td>
<td>4.10 ± 0.13</td>
<td>3.82 ± 0.12</td>
</tr>
<tr>
<td>ADPR</td>
<td>0.30 ± 0.05</td>
<td>0.46 ± 0.11</td>
<td>0.47 ± 0.06</td>
</tr>
<tr>
<td>NADP</td>
<td>0.331 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.33 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means (± S.E.M., n = 6 in each group). *P < 0.05 vs. A.
References


