Xanthine Oxidoreductase Activity in Perfused Hearts of Various Species, Including Humans

Jan Willem de Jong, Peter van der Meer, A. Selma Nieukoop, Tom Huizer, Rutger J. Stroeve, and Egbert Bos

Oxygen free radicals generated by xanthine oxidase have been implicated in cardiac damage. The activity of xanthine oxidase/reductase in adult rat heart is considerable. Its assay gives controversial results for other species, for example, rabbits and humans. Therefore, we perfused isolated hearts of various species, including explanted human hearts, to measure the conversion of exogenous hypoxanthine to xanthine and urate. We assayed these purines with high-performance liquid chromatography. The apparent xanthine oxidoreductase activities, calculated as release of xanthine plus 2× urate, were (milliunits per gram wet weight, mean±SEM) mice 33±3 (n=5), rats 28.5±1.4 (n=9), guinea pigs 14.4±1.0 (n=5), rabbits 0.59±0.09 (n=5), pigs <0.1 (n=6), humans 0.31±0.04 (n=7), and cows 3.7±0.8 (n=4). In rabbit heart the conversion of hypoxanthine to xanthine was slow, and that of xanthine to urate was even slower. On the other hand, guinea pig and human heart released little xanthine, indicating that xanthine breakdown exceeds its formation. We conclude that isolated perfused mouse, rat, guinea pig, and also bovine hearts show considerable xanthine oxidoreductase activity, contrastingly rabbit, porcine, and diseased human hearts. (Circulation Research 1990;67:770–773)

In heart tissue, adenine nucleotides are broken down to adenosine, inosine, and hypoxanthine. These can be found in the cardiac effluent and can be used as markers for ischemia.1 In rat heart, xanthine oxidoreductase catabolizes hypoxanthine to xanthine and urate.2,3 Adult rat heart shows considerable activity of the enzyme,4–8 in contrast to neonatal heart.3,9 The oxidase form, which generates free oxygen radicals,8 could potentially damage cardiac tissue. Whether the enzyme expresses itself in the hearts of humans6,8,10–14 and several other species, including rabbits,5–7,9,10 is controversial. The classical assay of the enzyme in homogenates may lead to erroneous results (e.g., because of endogenous inhibitors).4,14 We decided to use the isolated, perfused hearts of various species, measuring the conversion of added hypoxanthine to xanthine and urate. This enabled us to estimate the cardiac xanthine oxidoreductase activity in a physiological environment.

Materials and Methods

Perfusion of Rodent Hearts

Fed adult animals (BALB/c and hybrid C57 black/ CBA mice, Wistar rats, Dunkin-Hartley guinea pigs, and New Zealand White rabbits) were anesthetized intraperitoneally with sodium pentobarbital in accordance with institutional guidelines. Hearts were removed, arrested, and perfused retrogradely with Tyrode’s buffer at 9.6 kPa and 37° C as described previously.1 Heart weight was 0.15–0.20 g (mouse), 0.9–1.6 g (rat), 1.6–2.2 g (guinea pig), and 8–12 g (rabbit). Cannulation of mouse aortas proved difficult and time consuming: about 25 minutes/aorta was needed. For the other species, 1 minute sufficed. In all hearts, except those from mice, apex displacement was used to monitor function and heart rate. Coronary flow was measured electromagnetically (Skalar, Delft, Netherlands) or by timed collection of perfusate. Unless otherwise indicated, a control period of 15 minutes was used; then, the perfusion medium was supplemented with hypoxanthine (Merck, Darmstadt, FRG), infused just above the aortic cannula, to give an optimal substrate concentration, usually 30–50 μM. In the coronary effluent, purines were assayed by high-performance liquid chromatography,1 with detection at 295 nm (urate) and 254 nm (hypoxanthine, xanthine). For each species, perfusions with [8-14C]hypoxanthine (5–40

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Perfusion of Human, Porcine, and Bovine Hearts

Hearts of transplant patients in end-stage heart failure caused by ischemic heart disease or dilated cardiomyopathy were arrested in situ with St. Thomas’ Hospital cardioplegic solution at 4°C.15 Hearts from anesthetized, young adult pigs (hybrid Yorkshire/Danish Landrace, fasted overnight) underwent cardioplegic arrest just before or after excision at the conclusion of experiments performed for other purposes. Bovine hearts were flushed with the cardioplegic solution (with 5,000 IU/l heparin [Organon Teknika Nederland BV, Boxtel, Nederland]) within 15–20 minutes after slaughter in a local abattoir.

Hearts were transported in ice-cold saline; cannulation time was 30–50 minutes. Then, retrograde perfusion of the aorta was started. The perfusion fluid (37°C) consisted of Tyrode’s buffer1 supplemented with 5 mM sodium pyruvate, 10 IU/l insulin (Novo, Industri AS, Bagsvaerd, Denmark), 5,000 IU/l heparin, and 25 g/l dextran (40,000 Da; Pharmacia, Uppsala, Sweden). After about 20 minutes of perfusion, 50 µM hypoxanthine was added. The perfusion fluid was oxygenated with 95% O₂–5% CO₂, using an S-070/S oxygenator (Shiley Laboratories Inc., Irvine, Calif.), with heat exchanger and defoaming membrane. The perfusion apparatus was also equipped with a roller pump, a manometer, an LPE-1440 filter (Pall BioSupport Corp., Glen Cove, N.Y.), a bubble trap, and a fluid reservoir. Blood was washed from the hearts for 10 minutes at a rate of 200 ml/min. Then, recirculation was started with 800–2,200 ml; perfusion pressure was 6.4–8.3 kPa. In human and porcine hearts, flow necessary to maintain perfusion pressure was 200–600 ml/min. Bovine hearts required 900–1,200 ml/min. Heart weight after the experiment was 300–800 g (humans), 200–300 g (pigs), and 1.9–3.2 kg (cows).

Results

Rodent Hearts

Because of their small size, we were unable to monitor function in mouse hearts. In the other hearts, changes in heart rate and apex displacement were minimal during the experiment. Control coronary flow (milliliters per minute per gram wet weight, mean±SEM) was 7.6±1.0 (mice), 7.3±0.5 (rats), 11.4±1.1 (guinea pigs), and 5±0.6 (rabbits). In the course of the experiment, flow decreased somewhat.

In contrast to its precursors,16 infused hypoxanthine had no obvious inotropic or chronotropic effects; it was not vasoactive. We measured the catabolites xanthine and urate in the coronary effluent. Xanthine concentrations (micromolar) amounted to 2.9±0.4 (mice), 2.4±0.2 (rats), 0.23±0.08 (guinea pigs), and 0.106±0.014 (rabbits); urate concentrations (micromolar) were 1.3±0.3, 1.19±0.09, 0.74±0.09, and 0.018±0.004, respectively. The studies with radioactive hypoxanthine excluded a major contribution of xanthine from guanine. We found no evidence of urate breakdown to allantoin.

The apparent xanthine oxidoreductase activity, calculated from the concentrations mentioned above, was high in mouse hearts (33 mU/g). The activity was comparable in rat hearts, twice lower in guinea pig hearts, and very low in rabbit hearts (Table 1). In the latter, the conversion of hypoxanthine to xanthine was very slow, and that of xanthine to urate was even slower (Table 1). Exogenous hypoxanthine affected only the first reaction step in rabbit hearts. The guinea pig hearts released relatively little xanthine.

Porcine, Bovine, and Human Hearts

Human and porcine hearts beat regularly, whereas bovine hearts showed only atrial activity. Coronary flow (milliliters per minute per gram wet weight) was 0.82±0.12 (humans), 1.8±0.2 (pigs), and 0.43±0.07 (cows). Xanthine and urate concentration increased per minute by 0.012±0.001 and 0.08±0.02 µM, respectively, in human heart perfusate and by 0.72±0.10 and 1.2±0.4 µM, respectively, in bovine heart perfusate. We observed only marginal changes in the perfusate of porcine heart.

From Table 1 it is clear that relatively little activity was present in explanted human hearts, that is, 0.31 mU/g. Only porcine hearts showed a lower value: the activity was below the detection limit (0.1 mU/g). Bovine hearts had a 10-fold higher activity and fivefold lower urate/xanthine ratio than human hearts.

To preserve the hearts of the larger species, we administered a cardioplegic solution. To check whether this affected the xanthine oxidoreductase activity, we subjected rat hearts (which display a relatively high activity) to cold cardioplegia/ischemia. Figure 1 shows that this procedure hardly affected the enzymatic activity. We also tested in rat hearts whether the perfusion fluid used for the large hearts

<table>
<thead>
<tr>
<th>Species</th>
<th>Xanthine (nmol/min/g)</th>
<th>Urate (nmol/min/g)</th>
<th>XOD (mU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>18±2</td>
<td>8.3±1.4</td>
<td>33±3</td>
</tr>
<tr>
<td>Rat</td>
<td>14.1±1.1</td>
<td>7.8±0.4</td>
<td>28.5±1.4</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>1.2±0.3</td>
<td>7.2±0.5</td>
<td>14.4±1.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.51±0.08</td>
<td>0.075±0.015</td>
<td>0.59±0.09</td>
</tr>
<tr>
<td>Pig</td>
<td>&lt;0.05</td>
<td>&lt;0.07</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Human</td>
<td>0.023±0.003</td>
<td>0.14±0.02</td>
<td>0.31±0.04</td>
</tr>
<tr>
<td>Cow</td>
<td>1.1±0.3</td>
<td>1.4±0.3</td>
<td>3.7±0.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM and per gram wet weight. Hypoxanthine was infused into isolated hearts. From the xanthine and urate production rates, the xanthine oxidoreductase (XOD) activity was calculated as xanthine+2× urate. n, Number of experiments.
influenced the enzyme. Again, a similar activity was found with both perfusion fluids. These control experiments make it unlikely that the low activity found in human, porcine, and bovine hearts is due to differences in experimental setup.

**Discussion**

**Species Differences**

Ronca-Testoni and Borghini were the first to show urate production by the isolated, perfused rat heart. We confirmed their observation that urate composes the major purine released from rat hearts under basal conditions. Gerlach et al. had similar observations in guinea pig hearts. We reported blockade of hypoxanthine breakdown during anoxia by allopurinol. These observations strongly suggested that rat and guinea pig hearts contain xanthine oxidoreductase. Enzymatic measurements confirmed this hypothesis. Reported discrepancies on the activity of cardiac xanthine oxidoreductase in various other species initiated the present study. The apparent xanthine oxidoreductase activity in mouse hearts (33 mU/g), measured with hypoxanthine, was of the same order of magnitude as that in rat and guinea pig hearts (Table 1). It exceeds the only reported value for mouse hearts by a factor of 10. Our estimate for rat and guinea pig hearts is in reasonable agreement with values found in extracts. The conversion of xanthine to urate seems to be faster in guinea pig than in mouse and rat hearts. This should be checked with xanthine as the substrate.

The activity found in isolated pig hearts was <0.1 mU/g. Several authors reported minimal activity of the enzyme in pig heart homogenates. Our value for cow hearts agrees with data given in References 13 and 21 but is >30× higher than that reported in Reference 22. In guinea pig and human hearts, xanthine breakdown seems to be faster than xanthine formation. Rabbit hearts had little xanthine oxidoreductase activity, as calculated from the xanthine and urate production. Several authors reported minimal xanthine oxidoreductase activity in rabbit heart homogenate, which is in line with our ex vivo observation. The high activity in homogenate found by Wajner and Harkness is puzzling, as is the high activity reported for human heart autopsies.

**Xanthine Oxidoreductase in Heart Explants**

Recently, we suggested that xanthine oxidoreductase is present in the hearts of patients suffering from ischemic heart disease. This was based on the demonstration of cardiac urate production during transient myocardial ischemia. Autopsy material revealed high activity of the enzyme. Limited histochemical work has shown substantial amounts of xanthine oxidoreductase in human heart endothelium. On the other hand, several authors have reported very low to undetectable activity of the enzyme in human hearts. Based on our isolated heart perfusions, we conclude that hearts from patients with cardiomyopathy or end-stage ischemic heart disease are almost devoid of xanthine oxidoreductase activity. We cannot exclude the possibility that the diseased human heart contains inactive xanthine oxidoreductase. Such enzyme has been described recently. Xanthine oxidase can destroy itself by self-generated O2 metabolites. At present, we assume that extracardiac factors, such as neutrophils or other blood components, are responsible for the apparent xanthine oxidoreductase activity observed in catheterized patients or autopsies.

**Conclusion**

Isolated, perfused mouse, rat, guinea pig, and also bovine hearts show considerable xanthine oxidoreductase activity, contrasting porcine, rabbit, and diseased human hearts.

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**References**

2. Ronca-Testoni S, Borghini F: Degradation of perfused adenine compounds up to uric acid in isolated rat heart. J Mol Cell Cardiol 1982;14:177–180
5. Grum CM, Ragsdale RA, Ketai LH, Shlafer M: Absence of xanthine oxidase or xanthine dehydrogenase in the rabbit myocardium. Biochim Biophys Res Commun 1986;141:1104–1108
10. Wajner M, Harkness RA: Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. Biochim Biophys Acta 1989;991:79–84

**KEY WORDS**  • hypoxanthine  • isolated heart  • species differences  • urate  • xanthine oxidase
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