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Urate Production by Human Heart

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**Thoraxcenter, Erasmus University Rotterdam, The Netherlands; † Department of Experimental Pediatrics, University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas, USA; ‡Department of Medicine and Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; §Department of Cardiology, Catharina Hospital, Eindhoven, The Netherlands*

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T. Huizer, J. W. de Jong, J. A. Nelson, W. Czarnecki, P. W. Serruys, J. J. R. M. Bonnier and R. Troquay. Urate Production by Human Heart. *Journal of Molecular and Cellular Cardiology* (1989) **21**, 691–695. Xanthine oxidoreductase has been demonstrated in the heart of various species. However, its presence in human heart is still debated. In the literature, high to undetectable levels have been reported. We studied the arterial-venous urate difference across the heart of patients undergoing both routine cardiac catheterization and percutaneous transluminal coronary angioplasty. Urate is the end product of the reaction catalysed by xanthine oxidoreductase. In 10 patients, studied before angioplasty, the plasma urate level in the great cardiac vein exceeded the arterial one by 26 ± 10 nmol/ml ($P = 0.028$). In a further 13 patients, urate production was maximal immediately after the last of four consecutive occlusions (23 ± 8 nmol/ml, $P = 0.018$) and concomitant with increased coronary sinus hypoxanthine levels. We conclude that xanthine oxidoreductase is probably present in the heart of patients, suffering from ischemic heart disease, and responsible for the increase in urate production during transient myocardial ischemia.

Key Words: Xanthine oxidase; Uric acid; Myocardium; Ischemia; Human; Coronary angioplasty.

Introduction

Xanthine oxidoreductase activity has been demonstrated in the myocardium of a number of species (see Schoutsen and De Jong, 1987). Limited data are available on the enzyme in human heart. Autopsy material indicates high xanthine oxidase activity (Krenitsky *et al.*, 1974; Wajner and Harkness, 1988). Histochemical techniques have shown large amounts of the enzyme in human heart endothelium (Jarasch *et al.*, 1986). On the other hand, several authors have reported very low to undetectable xanthine oxidoreductase activity in human heart (Ramboer, 1969; Eddy *et al.*, 1987; Muxfeldt and Schaper, 1987). Preliminary observations assessing cardiac urate production in patients during pacing stress test at the University of Alabama (Nelson *et al.*, 1977) and in patients during coronary angiography in the National Institute of Cardiology, Warsaw (Czarnecki, 1988) have suggested that the human heart may be capable of urate production. We present evidence which shows that the human heart can produce significant amounts of urate. This observation suggests that a cardiac xanthine oxidoreductase is active in patients with ischemic heart disease.

Methods

Patients

Two studies were performed in patients, catheterized for percutaneous transluminal coronary angioplasty (PTCA). In 10 patients the urate concentrations of arterial and great cardiac vein plasma, obtained before PTCA,

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TABLE 1. Clinical characteristics of the study groups

Variable	Group 1 $n = 10$	Group 2 $n = 13$
Age (year), average	58	58
range	49 to 66	43 to 72
Gender (male/female)	7/3	10/3
CCS grade	III to IV	II to IV
Average severity of stenosis (%)		
before PTCA	78	79
after PTCA	44	37

CCS = Canadian Cardiovascular Society.

were assayed retrospectively (Group 1). Subsequently, in a prospective study (Group 2, 13 patients), urate and hypoxanthine concentrations were measured in arterial and coronary sinus plasma before, during and after angioplasty. In both studies, arterial blood was taken from the femoral artery. Great cardiac vein blood was sampled via the distal opening of a Webster flow catheter (Group 1) and coronary sinus blood via a diagnostic catheter (Group 2). All patients had a proximal stenosis < 1 cm from the origin of the left anterior descending artery and no collateral filling to the region supplied by the artery, seen at angiography. Amipaque or Isopaque contrast agents (Nyegaard, Oslo, Norway) were used for angiography. In all patients, vasoactive substances, except short-lasting nitrates, were discontinued at least 12 h before the study. The clinical characteristics are listed in Table 1.

Assays

To prepare plasma, blood was mixed in a heparinized tube with an equal volume of ice-cold 154 mM NaCl, containing 20 μM dipyridamole (Boehringer, Ingelheim, GFR) and 10 μM erythro-9-(2-hydroxy-3-nonyl)adenine (Wellcome, London, UK). These drugs were used to inhibit adenosine uptake and breakdown (Ontyd and Schrader, 1984; Edlund *et al.*, 1985). The plasma was kept at −80°C. Deproteinization was carried out with an equal volume of 8% $HClO_4$ (w/v) and the supernatant fraction neutralized with 2 M KOH/1 M K_2CO_3. HPLC-determination of urate and hypoxanthine concentrations in the plasma extract were performed on a μBondapak C_{18} column. A 100 μl sample was eluted with a mixture of CH_3OH (100 ml) and KH_2PO_4 (10 g/l, 1000 ml), pH 5-7, at a flow

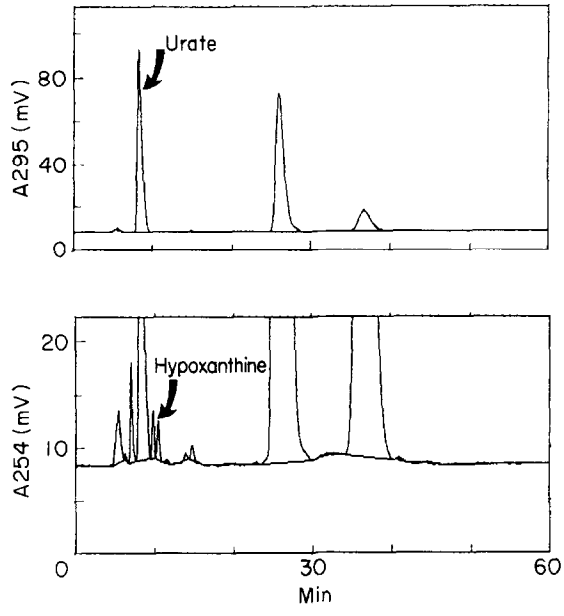

FIGURE 1. High-performance liquid chromatography of plasma extract. Urate was detected at 295 nm, hypoxanthine at 254 nm. The contrast agent used showed up in the chromatogram, but did not interfere with the peaks of interest.

TABLE 2. Arterial and venous urate levels before, immediately after four dilations, and during recovery

Patient no.	Before PTCA		After 1st dilation		After 2nd dilation		After 3rd dilation		After 4th dilation		Recovery	
	ART	CS	ART	CS	ART	CS	ART	CS	ART	CS	ART	CS
1	217	211	214	217	211	217	218	216	212	217	215	218
2	236	216	224	184	216	195	213	207	213	200	202	196
3	224	229	223	220	218	215	218	219	217	217	215	211
4	279	291	280	290	279	281	236	292	245	284	262	282
5	242	254	231	266	239	257	244	260	200	266	230	253
6	216	224	219	221	208	216	211	218	209	216	202	206
7	252	261	260	252	258	258	244	262	253	245	244	240
8	449	495	457	481	481	478	455	480	469	512	462	469
9	196	188	185	181	179	182	177	178	175	174	168	173
10	230	242	218	214	201	207	197	209	191	204	187	197
11	292	278	264	268	271	299	266	281	272	283	239	264
12	239	207	241	191	234	224	220	230	184	238	225	221
13	192	268	208	244	253	208	253	279	174	251	226	239
Mean	251	259	248	248	250	249	242	256	232	254	237	244
S.E.M.	18	21	19	22	21	21	19	21	21	23	20	21

ART = arterial; CS = coronary sinus. Data are in nmol/ml.

rate of 0.6 ml/min. The column was equipped with a LC-18 guard-column (Supelco, Bellefonte, PA). The Waters-HPLC equipment consisted of: WISP 710B cooled autosampler, Model 6000A pump, Model 490 multi wavelength detector, and Model 840 computer. Peaks were identified by retention times, internal standards and enzyme shifts. The optimal wavelengths for urate and hypoxanthine detection proved to be 295 and 254 nm as at these levels adsorption was maximal and disturbance by other materials minimal (Fig. 1). Sample preparation and assay were based on earlier work (Harmsen *et al.*, 1981). In 27 arterial and venous plasma samples of Group 2, urate was also assayed spectrophotometrically with uricase according to Scheibe *et al.* (1974). Enzyme was provided by Boehringer (Mannheim, GFR). Comparison of the data obtained with both methods showed that they correlated closely.

Data presented were analysed with Student's *t*-test for paired variates, or, where appropriate with two-way analysis of variance. A P value of <0.05 was considered as significant. The correlation test was done according to Bland and Altman (1986).

Results

In the preliminary studies, mentioned in the Introduction, hearts produced urate. In the American study, the arterial and venous blood urate levels were 59 ± 20 and 120 ± 23 nmol/ml, resp. ($n = 7$, $P = 0.003$). In the Polish study, these values were 96 ± 15 and 145 ± 25 nmol/ml resp. ($n = 6$, $P = 0.028$).

In Group 1, all patients had an isolated proximal left anterior descending artery stenosis and angina pectoris. In this group the arterial urate concentration was significantly lower than the coronary venous one (216 ± 17 and 242 ± 17 nmol/ml); a difference of 26 nmol/ml ($P = 0.028$). In seven out of 10 patients, the heart produced urate.

In a comparable patient population (Group 2, see Table 2), plasma urate concentrations were similar to those of Group 1, but the arterio-venous difference before coronary angioplasty was relatively small. Consequently we were unable to demonstrate significant urate production *before* coronary angioplasty. Analysis of data showed a significant increase in urate production during balloon inflations ($F = 2.85$; $P < 0.05$). *After* the third and fourth inflations, venous urate levels were significantly higher than arterial ones (Fig. 2). They differed 14 nmol/ml ($P = 0.009$) and 23 nmol/ml ($P = 0.018$), resp. Even after 15 min of recovery, urate production was still significant. The difference was 7 nmol/ml ($P = 0.033$).

In the latter study we also measured the arterial and coronary sinus hypoxanthine levels with HPLC. The arterial hypoxanthine

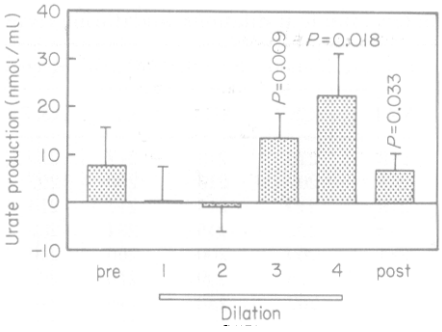

FIGURE 2. Urate production by the heart of 13 patients with single left anterior descending coronary artery stenosis, before coronary angioplasty (pre), after each balloon deflation (dilation one to four) and after 15 min of recovery (post). Mean coronary venous-arterial values are given with 1 S.E.M. Significant urate production was found immediately after the last two dilations, and during recovery.

plasma concentration slightly exceeded the venous one before angioplasty (0.58 ± 0.07 and 0.42 ± 0.07 nmol/ml, respectively, $P = 0.015$). Immediately following PTCA these values were 0.32 ± 0.06 and 1.28 ± 0.19 nmol/ml, respectively, $P < 0.001$ (average of four attempts). Thus cardiac uptake turned into production. Fifteen minutes after angioplasty arterial plasma hypoxanthine levels were not different from the venous ones.

Discussion

Xanthine oxidoreductase activity is detectable in the heart of a number of species (for reviews, see Schoutsen and De Jong, 1987; Downey *et al.*, 1988). In pig heart it seems to be absent (Podzuweit *et al.*, 1986; Muxfeldt and Schaper, 1987). In rabbit heart both Schoutsen *et al.* (1983) and Chambers *et al.* (1985) were unable to demonstrate the enzyme, but Wajner and Harness (1988) measured high activity. The literature on xanthine oxidoreductase in human heart is also conflicting. The reports vary from high (Krenitsky *et al.*, 1974; Jarasch *et al.*, 1986; Wajner and Harness, 1988) to (very) low levels (Watts *et al.*, 1965; Ramboer, 1969; Eddy *et al.*, 1987; Muxfeldt and Schaper, 1987). We want to emphasize that in these reports the number of samples assayed was often very small. Muxfeldt and Schaper (1987) found very low amounts of xanthine oxidoreductase in the two human heart biopsies studied. Krenitsky *et al.* (1974) reported data on one autopsy sample. These authors observed enzyme activity with ferricyanide as the electron acceptor but did not use NAD or oxygen as the cosubstrate. Allopurinol inhibited the activity. Eddy *et al.* (1987) could not demonstrate xanthine oxidoreductase in human ventricular tissue. Supposedly the four biopsies studied were not taken from ischemic hearts.

A possible explanation for the discrepancies in activity found could be a difference in quality of the hearts examined. Our data indicate that the enzyme could be active in the human heart *in vivo*. We cannot exclude that the urate production measured originated from xanthine oxidoreductase activity in polymorphonuclear neutrophils, adhering to areas of the coronary endothelium that are injured by the balloon during inflation.

In the American and Polish studies, mentioned before, *blood* was deproteinized with $HClO_4$ which causes a partial loss during sample clean-up. Never the less the arteriovenous differences in urate were significant. Moreover, they suggested that patients with a more severe ischemic heart disease produced the highest amounts of urate. In the American study, patients experiencing pain during a pacing stress test released lactate and showed the highest urate production. Czarnecki (1988) observed that patients with a history of subendocardial infarction produced high amounts of urate whereas patients with normal myocardium or extensive myocardial damage produced less. Our present results support this idea. Group 1, which comprised patients with CCS grades III and IV, showed significant urate production before PTCA. Group 2, in which four out of 13 patients were CCS grade II, only started to produce significant amounts of urate after several dilations. After each of the angioplasty attempts, venous plasma hypoxanthine [the relatively stable substrate for xanthine oxidoreductase (Harkness, 1988)] increased fourfold. The data suggest that ischemic myocardium at risk of infarction produces urate.

Patients of Group 1, all with a proximal stenosis of the left anterior descending coronary artery produced urate (Table 1). It is likely that this urate production was partly

due to endothelial damage, caused by insertion of the guide wire and the balloon catheter. In Group 2 urate production, which was not significant before PTCA, became obvious after repetitive angioplasty attempts (Fig. 2). Presumably, this is due to cardiac ATP breakdown, with a concomitant rise in hypoxanthine as a result of myocardial ischemia due to coronary occlusion by balloon inflation (see also Serruys *et al.*, 1989). Hypoxanthine serves as a substrate for xanthine oxidoreductase. We suggest that the human heart may contain active xanthine oxidoreductase.

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