Biochemical Pharmacology, Vol. 36, No. 13, pp. 2101–2107, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF ALLOPURINOL ON MYOCARDIAL ISCHEMIC INJURY INDUCED BY CORONARY ARTERY LIGATION AND REPERFUSION

DAVID V. GODIN* and SHABIR BHIMJI

Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

(*Received* 12 *May* 1986; *accepted* 24 *November* 1986)

Abstract—The effects of allopurinol pretreatment (1 mg/ml in the drinking water for 7 days at an estimated daily dose of 75 mg/kg) on biochemical and chemical changes occurring following left circumflex coronary artery ligation (40 min) and reperfusion (60 min) were examined in pentobarbital-anesthetized rabbits. During the ischemic phase, allopurinol pretreatment provided significant preservation of cellular ATP levels and of mitochondrial ATP generation as compared with untreated animals ($P < 0.05$). During the reperfusion phase, allopurinol pretreatment significantly prevented the decrease in left ventricular pressure, sodium and calcium accumulation and decreases in sarcolemmal Na^+, K^+-stimulated and sarcoplasmic reticulum K^+, Ca^{2+}-stimulated ATPase activities as compared with untreated animals ($P < 0.05$). In contrast, the decrease in mitochondrial (azide-sensitive) ATPase during ischemia and the partial recovery during reperfusion were unaffected by allopurinol pretreatment. Our results indicate that the myocardial protective effects of allopurinol may differ mechanistically in the ischemic and reperfusion phases of injury. The fact that rabbit hearts do not contain detectable xanthine oxidase activity would seem to preclude an obligatory role of this enzyme both in the generation of myocardial ischemic/reperfusion injury and in the protective actions of allopurinol.

In-hospital deaths in patients with acute myocardial infarction result predominantly from two causes—primary arrhythmias and pump failure. Whereas the incidence of death due to arrhythmias has been reduced by the use of antiarrhythmic agents, the mortality rate after myocardial failure, manifested by cardiogenic shock or pulmonary edema or both, has not been altered substantially by currently available pharmacological interventions and remains high. The quantity of viable contracting myocardium in patients dying as a result of pump failure is less than that of patients in whom ventricular arrhythmias are the cause of death [1–3]. A therapeutic intervention decreasing the amount of myocardial tissue undergoing irreversible damage after coronary artery occlusion may not only reduce immediate mortality, but also leave the patient with a greater quantity of viable myocardium, thereby reducing the risk or extent of pump failure [4].

Since it was first established that the mass of damaged myocardium following coronary artery occlusion in man is a major determinant of prognosis, measurements of infarct size have been used extensively in evaluating interventions which may have a potentially beneficial effect on the course of myocardial ischemic injury [5, 6] Within the last decade, several drug interventions, such as glucose-insulin-potassium [7], hyaluronidase [8], β-blockers [9, 10] and calcium antagonists [11] have been studied for their effectiveness in protecting the ischemic myocardium. Of the numerous pharmacological interventions which are currently undergoing critical scrutiny, the xanthine oxidase inhibitor allopurinol appears to be promising in exerting a salutary influence on the ischemic myocardium [12–15].

Work by DeWall *et al.* [12] and Arnold *et al.* [13] has indicated that pretreatment of dogs with allopurinol provides protection against ischemic injury induced by coronary artery ligation. However, the criteria for effectiveness of allopurinol were largely based on hemodynamic and ECG measurements which do not readily permit the elucidation of the molecular mechanisms responsible for the observed protective effects. We have shown recently that allopurinol administered chronically in drinking water (at a concentration of 1 mg/ml) affords marked protection against myocardial ultrastructural alterations induced by coronary artery ligation and subsequent reperfusion in the rabbit [16], a species claimed to be devoid of measurable myocardial xanthine oxidase activity [17]. To further explore the cellular basis of allopurinol action on the ischemic myocardium, we have studied its effects on the maintenance of ionic balance, high energy phosphates, mitochondrial function and various subcellular organelle marker enzyme activities in anesthetized rabbits subjected to reversible coronary artery ligation.

MATERIALS AND METHODS

Male New Zealand white rabbits (2 to 2.5 kg) were randomly divided into four groups of ten animals

* Address correspondence to: Dr. David V. Godin, Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., V6T 1W5.

each as follows: *Group I*, sham-operated control animals that received no drug treatment; *Group II*, sham-operated allopurinol-treated animals; *Group III*, control animals subjected to coronary artery ligation and reperfusion: and *Group IV*, allopurinol-treated animals undergoing ligation and reperfusion. The duration of coronary ligation was 40 min and that of reperfusion was 60 min as described in earlier studies from our laboratory [16, 18]. All treated animals received allopurinol (1 mg/ml in drinking water) for 7 days prior to surgery—a treatment regimen which we have shown recently to be protective against myocardial ultrastructural damage following coronary artery ligation and reperfusion [16].

Coronary ligation. After the appropriate treatment, each animal was anesthetized with sodium pentobarbital (30 mg/kg) administered intravenously and prepared for coronary artery ligation as described previously [18]. If fibrillation occurred during the ischemic or reperfusion periods, sinus rhythm was restored by the application of 0.5 Watt-second countershocks, a level below the threshold shown by Koning *et al.* [19] to produce tissue damage in isolated rabbit hearts. Sham-operated animals (Groups I and II) were subjected to all experimental procedures, except that the left circumflex coronary artery was not occluded.

Chemical analyses. Samples for ATP analyses were obtained directly from the beating heart, quickly frozen in liquid nitrogen and assayed later according to the method of Jaworek *et al.* [20]. Intracellular sodium in the occluded zone was measured by the method of Moore *et al.* [21] and calcium, magnesium and potassium were determined by the method of James and Roufogalis [22].

Mitochondrial ATP generation. Mitochondria from control or ischemic tissue were isolated as described by Peng *et al.* [23]. ATP-generating capacity was measured by incubating freshly isolated mitochondria at 37° in a reaction medium containing 250 mM sucrose, 12.5 mM Tris-Hepes* (pH 7.2), 3.0 mM Tris-glutamate and 3.0 mM potassium dihydrogen phosphate. Mitochondria were added to provide a final concentration of 2.5 mg protein/ml, and the reactions were started by adding ADP to a final concentration of 2.5 mM. After 15, 30, 45, 60 or 120 sec, the reactions were terminated by adding 10% cold trichloroacetic acid, and the ATP generated was measured as described above.

Lysosomal enzymes. Lysosomes from the occluded zone were isolated as previously described by Godin *et al.* [24]. The activities of three lysosomal hydrolases (acid phosphatase, cathepsin D and *N*-acetyl-β-glucosaminidase) were measured as described by Barett [25].

Marker enzyme activities. Fractionation of the left ventricle to yield mitochondria- and sarcolemma-enriched vesicle preparations was done as previously described [24]. To minimize the effects of vesiculation on measured enzyme activities [26], assays were performed in the presence of experimentally determined, maximally activating concentrations of Triton X-100 (0.005% for the mitochondrial and sarcoplasmic reticular enzymes and 0.01% for the sarcolemmal enzymes). Protein was determined by the method of Lowry *et al.* [27] using bovine serum albumin as a standard.

Sufficient ischemic tissue was available from each animal for preparation of mitochondrial and sarcolemma-enriched fractions so that pooling of tissue from different animals was not required. The activity of cytochrome *c* oxidase was measured in both sarcolemmal and mitochondrial fractions to assess the mitochondrial contamination of the sarcolemmal preparation, and no differences were observed between control and ischemic-reperfused membranes (control 12.7 ± 3.1, ischemic reperfused 10.8 ± 3.4% contamination; mean ± SD, N = 7). Thus, ischemia-related changes in mitochondrial and sarcolemmal enzyme activities are not likely due to differences in subcellular fractionation. Bersohn *et al.* [28] have also shown that there was no difference in the fractionation of sarcolemma from control versus ischemic myocardial tissue. Finally, we have analyzed homogenates of control, ischemic and ischemic/reperfused myocardial samples for the presence of xanthine oxidase activity using the preparative procedure outlined by Chambers *et al.* [29] and the enzyme assay described by Rowe and Wyngaarden [30].

Statistical analysis. Results were analyzed using a one-way analysis of variance followed by Tukey's test to assess specific group differences at $P < 0.05$.

RESULTS

Because allopurinol had to be dissolved in 5 N NaOH, and high concentrations of allopurinol were required, the drug was given in the drinking water (subcutaneous injections of high doses of allopurinol dissolved in NaOH caused severe irritation and, therefore, this method of administration was avoided). All the allopurinol-treated animals consumed approximately 180 ± 20 ml water/24 hr (giving an estimated daily allopurinol dose of approximately 75 ± 8 mg/kg). The food consumption and body weight of the allopurinol-treated animals were not affected by the allopurinol treatment (Table 1).

Occluded zone. The size of the occluded zone was similar in both the untreated and allopurinol-treated animals (untreated 60 ± 2, allopurinol-treated 62 ± 3% of left ventricle). Thus, any beneficial effects of allopurinol treatment cannot be ascribed to differences in the amount of ischemic tissue at risk of undergoing necrosis.

Table 1. General features of allopurinol-treated animals

Treatment	Body wt (kg)	Food consumption (g/24 hr)	Water consumption (ml/24 hr)
Control	2.8 ± 0.2	175 ± 20	200 ± 20
Allopurinol	2.9 ± 0.2	150 ± 15	180 ± 20

Allopurinol was administered in the drinking water at a concentration of 1 mg/ml for 7 days. Values are expressed as mean ± SE (N = ten animals).

* Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; and EGTA, ethyleneglycolbis (amino-ethylether)tetra-acetate.

Table 2. Hemodynamics following 40 min occlusion (40 min O) and 60 min reperfusion (60 min R) of the left circumflex coronary artery in allopurinol-treated and untreated animals

	Left ventricular pressure (mm Hg)	Left ventricular end-diastolic pressure (mm Hg)	Heart rate (beats/min)	Mean arterial pressure (mm Hg)	Rate of rise of left ventricular pressure (mm Hg/sec)
Untreated					
Preligation	100 ± 10	4 ± 2	290 ± 5	100 ± 10	4200 ± 200
40 min O	100 ± 15	6 ± 4	300 ± 10	90 ± 10	3800 ± 300
60 mm R	50 ± 5*	10 ± 5	315 ± 10*	50 ± 5*	1800 ± 200*
Allopurinol-treated					
Preligation	115 ± 10	5 ± 2	290 ± 10	120 ± 10	4300 ± 200
40 min O	110 ± 10	5 ± 2	290 ± 10	110 ± 10	4000 ± 200
60 min R	70 ± 10*†	10 ± 5	315 ± 10	70 ± 10*†	2300 ± 200*

Values are expressed as mean ± SE (N = ten animals).
* $P < 0.05$, significantly different from respective untreated or allopurinol-treated preligated value.
† $P < 0.05$, significantly different from corresponding untreated reperfusion value.

Table 3. Myocardial cations in untreated and allopurinol-treated animals following 40 min occlusion (40 min O) and 60 min reperfusion (60 min R)

	Sodium	Potassium (ng atoms/mg dry weight)	Magnesium (ng atoms/mg dry weight)	Calcium
Untreated				
Preligation	48.2 ± 8.1	177 ± 14	18.2 ± 1.1	2.6 ± 0.2
40 min O	79 ± 8*	226 ± 19*	22 ± 2	3.0 ± 0.2
60 min R	127 ± 11.6*	129 ± 11*	15.1 ± 1.6*	13.2 ± 1.9*
Allopurinol-treated				
Preligation	51 ± 8.7	210 ± 11	19.3 ± 2.1	3.0 ± 0.2
40 min O	69 ± 7*	236 ± 9	24 ± 4	3.1 ± 0.6
60 min R	91 ± 6.3*†	164 ± 9*†	14.6 ± 3.1*	6.8 ± 2.1*†

Values are expressed as mean ± SE (N = ten animals).
* $P < 0.05$, significantly different from respective untreated or allopurinol-treated preligated value.
† $P < 0.05$, significantly different from corresponding untreated reperfusion value.

Hemodynamics. Prior to coronary artery ligation, the hemodynamic status of allopurinol-treated animals was not detectably different from that of controls. Following occlusion and reperfusion, untreated animals showed a depression of both mean arterial and left ventricular pressures (Table 2). In the allopurinol-treated animals, mean arterial and left ventricular pressures were decreased to a similar degree following occlusion and reperfusion, but the magnitude of the depression was significantly less than that in the untreated animals. In both the allopurinol-treated and untreated animals, the left ventricular end-diastolic pressure did show a tendency to increase, but the increase was not significant when compared to preligated values. Allopurinol treatment did not alter significantly the increased heart rate and the decreased rate of rise of left ventricular pressure observed following coronary occlusion and reperfusion.

Ions. No significant differences were observed in the Na^+, K^+, Mg^{2+} or Ca^{2+} myocardial levels in control versus allopurinol-treated animals prior to or following 40 min of coronary artery ligation (Table 3). Reperfusion in untreated animals was associated with significant increases in myocardial calcium and sodium and significant decreases in potassium and magnesium. The reperfusion-induced alterations in myocardial sodium, potassium and calcium levels were reduced significantly by allopurinol treatment (Table 3).

Subcellular organelle enzymes. The activities of various subcellular organelle enzyme markers in both untreated and allopurinol-treated animals were similar prior to ligation (Table 4). Following ligation, the mitochondrial (azide-sensitive) ATPase activity decreased by the same extent (60%) in both groups of animals and showed partial recovery following reperfusion, which although somewhat greater in the allopurinol-treated animals was not significantly different from untreated animals. The reduction in sarcolemmal (ouabain-sensitive) Na^+, K^+-ATPase activity after occlusion was comparable in the untreated and the allopurinol-treated animals. Following reperfusion, the sarcolemmal ATPase activity was preserved to a greater degree in the allopurinol-treated animals (Table 4). Although sarcoplasmic reticulum (azide-insensitive, EGTA inhibitable) K^+,Ca^{2+}-ATPase activity decreased to the same extent in both the untreated and allopurinol-treated animals after 40 min of occlusion, following reper-

Table 4. Subcellular organelle ATPase activities in untreated and allopurinol-treated animals following ligation (40 min O) and reperfusion (60 min R)

	Mitochondrial ATPase	Sarcolemmal ATPase (μmol P_i/mg protein/hr)	Sarcoplasmic reticular ATPase
Untreated			
Preligation	224 ± 11	7.9 ± 0.3	3.3 ± 0.2
40 min O	92 ± 7*	5.1 ± 0.4*	2.2 ± 0.3*
60 min R	165 ± 7.1*	3.8 ± 0.4*	1.7 ± 0.3*
Allopurinol-treated			
Preligation	218 ± 7	8.1 ± 0.2	3.4 ± 0.2
40 min O	106 ± 9*	5.9 ± 0.3*	2.6 ± 0.3*
60 min R	175 ± 11*	4.9 ± 0.4*†	2.4 ± 0.2*†

Values are expressed as mean ± SE (N = ten animals).
* $P < 0.05$, significantly different from respective untreated or allopurinol-treated preligated value.
† $P < 0.05$, significantly different from corresponding untreated reperfusion value.

fusion a further decline in the activity of this enzyme was observed only in the untreated animals (Table 4).

ATP. Myocardial ATP levels following coronary artery ligation and reperfusion in untreated and allopurinol-treated animals are shown in Fig. 1. The mean level of myocardial ATP prior to ligation, although somewhat higher in the allopurinol-treated animals, was not significantly different from that observed in untreated animals. Following 40 min of coronary artery ligation, control animals showed a 35% drop in myocardial ATP content followed by a further 48% decrease following reperfusion. In marked contrast, ATP levels in the allopurinol-treated animals were well conserved. There was only a 7% decrease after 40 min of ligation and a further 7% drop in ATP level following 60 min in reperfusion.

Mitochondrial ATP generation. The results of mitochondrial ATP generation are summarized in Table 5. Prior to ligation no difference in mitochondrial ATP generation was observed between untreated and allopurinol-treated animals. Following 40 min occlusion, untreated animals showed a marked inhibition of mitochondrial ATP-generating capacity (80%) which had decreased further (to greater than 90% inhibition) after reperfusion. Allopurinol pretreatment produced significant protective effects, particularly in the ischemic phase (where inhibition was only 45%) as compared with the reperfusion phase (where 65% inhibition was observed).

Lysosomal enzymes. The non-sedimentable fraction of the lysosomal enzymes *N*-acetyl-β-glucosaminidase and acid phosphatase increased significantly in both the untreated and allopurinol-treated animals following ligation and reperfusion. The increase in the non-sedimentable fraction of

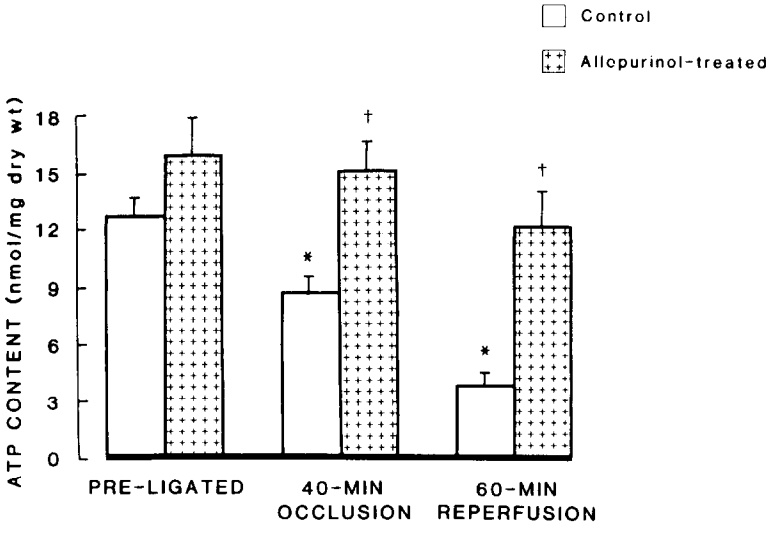

Fig. 1. Myocardial ATP levels in control and allopurinol-treated animals following coronary artery occlusion (40 min) and reperfusion (60 min). Key: *$P < 0.05$, significantly different from corresponding control preligated value; and †$P < 0.05$, significantly different from corresponding control occlusion or reperfusion value.

Table 5. Mitochondrial ATP generation in hearts of untreated and allopurinol-treated animals subjected to 40 min occlusion and 60 min reperfusion

	ATP generation (μmol/mg/min)	
	Untreated	Allopurinol-treated
Preligation	0.61 ± 0.06	0.59 ± 0.09
40-min Occlusion	0.12 ± 0.03*	0.36 ± 0.08†
60-min Reperfusion	0.05 ± 0.02*	0.22 ± 0.07†

Values are expressed as mean ± SE (N = ten animals)
* $P < 0.05$, significantly different from respective untreated or allopurinol-treated preligated value.
† $P < 0.05$, significantly different from corresponding untreated occluded or reperfusion value.

to the protective effects of allopurinol in myocardial ischemic/reperfusion injury in dogs [13, 15, 29, 31, 32] and in rats [14]. In all the foregoing studies, allopurinol was given chronically for at least 24 hr prior to, and in some cases also during, the induction of ischemia. Reimer and Jennings [33], on the other hand, failed to demonstrate protection by allopurinol in dogs when the drug was administered immediately prior to coronary occlusion. In a recently completed study, we have shown that rabbits subjected to the same allopurinol treatment regimen as the one used here showed marked preservation of myocardial ultrastructure following coronary artery ligation and reperfusion, with particularly prominent effects on mitochondrial integrity [16]. The results of the present biochemical study reinforce these observations, in that allopurinol pretreatment protected against the marked reduction in mitochondrial

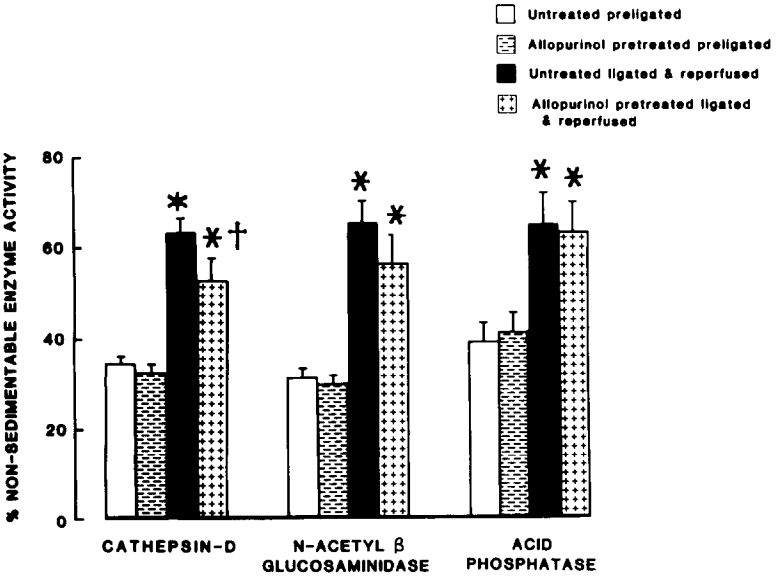

Fig. 2. Non-sedimentable activities of lysosomal enzymes, acid phosphatase, cathepsin D and *N*-acetyl-β-glucosaminidase, following coronary ligation and reperfusion in untreated and allopurinol-treated animals. Lysosomal enzyme activity is expressed as percent non-sedimentable over sedimentable plus non-sedimentable. Key: *$P < 0.05$, significantly different from preligated value; and †$P < 0.05$, significantly different from corresponding untreated occlusion or reperfusion value.

cathepsin D was significantly less in the allopurinol-treated animals (Fig. 2).

Myocardial xanthine oxidase activity. Consistent with a previous report in the literature [17], we have been unable to detect xanthine oxidase activity in homogenates of rabbit myocarium—even following a 40-min period of ischemia with subsequent reperfusion for 60 min, conditions under which maximal increases in the activity of xanthine oxidase would have been expected [29].

DISCUSSION

The possibility that allopurinol may reduce the severity of ischemic injury following coronary artery occlusion was first suggested by DeWall *et al.* [12] on the basis of experiments involving dogs and sheep subjected to coronary artery ligation. Since that time, a number of reports have been published attesting

ATP-generating capacity and depletion of cellular ATP levels associated with myocardial ischemic/reperfusion injury. The beneficial effects of allopurinol were not restricted, however, to mitochondria but were extended to the sarcolemma (as indicated by reduced Na^+,K^+-ATPase inactivation and preservation of myocardial cation composition), the sarcoplasmic reticulum (as seen in the activity of the Ca^{2+}-dependent ATPase associated with this organelle) and, to some degree, cathepsin D-containing lysosomes. Certain protective actions of allopurinol, notably the preservation of cellular ATP levels and of mitochondrial ATP-generating capacity, were exerted predominantly in the ischemic phase, while other beneficial actions, such as the prevention of a decrease in left ventricular pressure, of sodium and calcium accumulation and of the decreases in sarcolemmal and sarcoplasmic reticulum ATPases, were greater during reperfusion. This

would seem to suggest that the molecular processes determining cellular injury, and therefore the protective actions of allopurinol, are mechanistically different in these two phases.

The ability of allopurinol to protect against ischemic injury in the heart [13–15, 29, 31, 32] and other tissues such as kidney [34] and skeletal muscle [35] has usually been attributed to its xanthine oxidase inhibiting properties. Some have postulated that xanthine oxidase inhibition might be protective by facilitating purine salvage, thereby enabling ischemic tissues to maintain their ATP stores [12, 13]. However, Chambers *et al.* [29] have suggested that the washout of nucleotide degradation products of ATP is so rapid ($T_{\frac{1}{2}} = 3$ min) that it is unlikely that allopurinol could preserve ATP by this mechanism. Our demonstration in the present study that allopurinol exerts marked ATP-sparing effects in the ischemic rabbit heart which is devoid of measurable xanthine oxidase activity further supports this view. On the basis of the biochemical data obtained here and the results of our earlier ultrastructural investigation [16], it seems likely that the preservation by allopurinol of ATP levels in ischemic myocardium is related to protective effects of the drug on the structural and functional integrity of mitochondria. The mitochondrial actions of allopurinol were also emphasized in the recent study by Peterson *et al.* [36] of globally ischemic rabbit myocardium showing that allopurinol can facilitate the transfer of electrons from ferrous iron to ferric cytochrome *c*, thereby enhancing electron transport under conditions of ischemia-induced perturbations of mitochondrial membrane components.

There is now a growing body of evidence which implicates oxygen-derived free radicals in the damage produced when ischemic tissues are subjected to reperfusion [37–41]. Free radicals are able to produce myocardial alterations characteristic of ischemic/reperfusion injury, including disruption of plasma membranes and lysosomes, damage to mitochondria and sarcoplasmic reticulum, increases in vascular permeability, and the initiation of ventricular arrhythmias [42–45]. Jolly *et al.* [46] have demonstrated that infusion into the left atrium of superoxide dismutase (which catalyzes the conversion of superoxide radicals to hydrogen peroxide plus molecular oxygen) and catalase (which degrades hydrogen peroxide to water) reduces the extent of ischemic injury in a canine occlusion-reperfusion model. However, more recent work by Werns *et al.* [47] demonstrating that superoxide dismutase alone protects reperfused ischemic myocardium suggests that tissue damage under these conditions may be largely determined by superoxide anion. One possible source of superoxide in the reperfused ischemic myocardium is the xanthine oxidase-catalyzed conversion of hypoxanthine (arising from the degradation of ATP) to uric acid—a process favored both by the reintroduction of oxygen and by the conversion of xanthine dehydrogenase to xanthine oxidase under these conditions [48, 49]. While inhibition of xanthine oxidase may contribute to the protective effects of allopurinol in ischemic/reperfused tissues containing measurable levels of enzyme activity, this does not seem to be a likely explanation in the case of rabbit myocardium which we and others [17] have shown lacks detectable xanthine oxidase activity.

It has been pointed out recently by Hallet *et al.* [50] that tissue contents of xanthine dehydrogenase and the ease with which it is convertible to the radical generating xanthine oxidase form do not seem to correlate with tissue sensitivity to post-ischemic reperfusion injury. This has led these investigators to explore polymorphonuclear leucocytes (PMN) as a possible source of reactive oxygen radicals during reperfusion. The ability of aprotinin, a protease inhibitor previously shown to protect against reperfusion-induced injury, to inhibit the overproduction of reactive oxygen metabolites by isolated PMN *in vitro* was taken as evidence for a possible role of PMN-derived oxygen radicals in the generation of reperfusion injury [50]. Similarly, the protective effects of nafazatrom in a canine model of ischemic/reperfusion injury have been attributed to its ability to interfere with neutrophil functional properties, including the generation of superoxide radicals [51]. This explanation does not, however, seem to extend to allopurinol. Jones *et al.* [52] have postulated that the levels of xanthine oxidase in neutrophils are insufficient to account for the flux of superoxide radicals associated with activation. This claim was based on the lack of demonstrable *in vitro* inhibition by allopurinol on neutrophil superoxide generation, chemotaxis or degranulation [52].

Thus, our data, when taken with the results of several other published reports, indicate that xanthine oxidase-mediated oxygen radical generation does not play an obligatory role in myocardial ischemic/reperfusion injury, and the protective effects of allopurinol against such injury are not primarily attributable to xanthine oxidase inhibition. Vartanyan and Gurevich [53] have reported that allopurinol administration is associated with a time-dependent increase in the superoxide dismutase activity of mouse liver. Preliminary experiments in our laboratory have shown that ischemic/reperfusion injury in rabbits is associated with a marked increase in susceptibility of myocardial homogenates to *t*-butylhydroperoxide-induced glutathione depletion and lipid peroxidation (D. V. Godin and M. E. Garnett, manuscript in preparation). Based on these observations and the aforementioned findings of Vartanyan and Gurevich, we are currently exploring the possibility that allopurinol pretreatment may afford protection against ischemic/reperfusion damage by enhancing the antioxidant capacity of myocardial tissues.

Acknowledgement—This study was funded by a grant from the British Columbia Heart Foundation.

REFERENCES

1. M. J. Davies, *Br. Heart J.* **45**, 88 (1981).
2. W. Schaper, *Ann. N.Y. Acad. Sci.* **382**, 69 (1982).
3. R. A. Kloner and E. Braunwald, *Cardiovas. Res.* **14**, 371 (1980).
4. M. J. Davies, W. F. M. Futton and W. B. Robertson, *J. Path.* **127**, 99 (1979).

5. P. R. Maroko and E. Braunwald, *Ann. intern. Med.* **79**, 720 (1973).
6. E. Braunwald and P. R. Maroko, *Circulation* **50**, 206 (1974).
7. M. K. Heng, R. M. Norris, T. Peter, H. D. Nisbet and B. N. Singh, *Cardiovas. Res.* **12**, 429 (1978).
8. A. S. Most, R. J. Capone and P. A. Mastrofrancesco, *Am. J. Cardiol.* **38**, 28 (1976).
9. A. Zeigelhoffer, P. K. Das, G. P. Sharma, P. K. Singal and N. S. Dhalla, *Can. J. Physiol. Pharmac.* **57**, 979 (1979).
10. D. J. Hearse, J. E. Baker and S. M. Humphrey, *J. molec. cell. Cardiol.* **12**, 733 (1980).
11. R. G. Wilcox, J. M. Roland, D. C. Banks, J. R. Hampton and J. R. A. Mitchell, *Br. med. J.* **280**, 885 (1980).
12. R. A. DeWall, K. A. Vasko, E. L. Stanley and P. Kezdi, *Am. Heart J.* **82**, 362 (1971).
13. W. L. Arnold, R. A. DeWall, P. Kezdi and H. J. Zwart, *Am. Heart J.* **99**, 614 (1980).
14. A. S. Manning, D. J. Coltart and D. J. Hearse, *Circulation Res.* **55**, 545 (1984).
15. J. R. Stewart, C. L. Crute, V. Loughlin, M. L. Hess and L. J. Greenfield, *J. thorac. cardiovasc. Surg.* **90**, 68 (1985).
16. D. V. Godin, S. Bhimji and J. H. McNeill, *Virchows Arch.* [Cell Path.], **52**, 327 (1986).
17. D. M. Yellon, V. Richard, D. J. Hearse, J. DeLeiris, M. Maxwell, P. Kithas, S. Frank, M. Artman and J. M. Downey, *Fedn Proc.* **44**, 1480 (1985).
18. M. M. Moore and D. V. Godin, *Can. J. Physiol. Pharmac.* **62**, 212 (1984).
19. G. Koning, A. H. Veefkind and H. Schneider, *Am. Heart J.* **100**, 473 (1980).
20. D. Jaworek, W. Gruber and H. U. Bergmeyer, in *Methods of Enzymatic analysis* (Ed. H. U. Bergmeyer), p. 2097. Verlag Chemie International, New York (1974).
21. R. D. Moore, J. W. Mumford and T. J. Pillsworth, *J. Physiol., Lond.* **388**, 277 (1983).
22. M. R. James and B. D. Roufogalis, *Can. J. Physiol. Pharmac.* **55**, 1190 (1977).
23. C. F. Peng, J. J. Kane, M. L. Murphy and K. D. Straub, *J. molec. cell. Cardiol.* **9**, 897 (1977).
24. D. V. Godin, J. M. Tuchek and M. M. Moore, *Can. J. Biochem.* **58**, 777 (1980).
25. A. J. Barett, in *Lysosomes: A Laboratory Handbook* (Ed. J. T. Dingle), p. 46. North Holland, Amsterdam (1972).
26. L. R. Jones, H. R. Besch Jr., J. W. Fleming, M. M. McConnaughey and A. M. Watanabe, *J. biol. Chem.* **254**, 530 (1979).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
28. M. M. Bersohn, K. D. Philipson and J. Y. Fukushima, *Am. J. Physiol.* **242**, C288 (1982).
29. D. E. Chambers, D. A. Parks, G. Patterson, R. Roy, J. M. McCord, S. Yoshida, L. F. Parmley and J. M. Downey, *J. molec. cell. Cardiol.* **17**, 145 (1985).
30. P. B. Rowe and J. B. Wyngaarden, *J. biol. Chem.* **241**, 5571 (1966).
31. D. A. Peterson, R. W. Asinger, K. J. Elsperger, D. C. Homans and J. W. Eaton, *Biochem. biophys. Res. Commun.* **127**, 87 (1985).
32. S. Akizuki, S. Yoshida, D. E. Chambers, L. J. Eddy, L. F. Parmley, D. M. Yellon and J. M. Downey, *Cardiovasc. Res.* **19**, 686 (1985).
33. K. A. Reimer and R. B. Jennings, *Circulation* **71**, 1069 (1985).
34. M. S. Paller, J. R. Hoidal and T. F. Ferris, *J. clin. Invest.* **74**, 1156 (1984).
35. R. J. Korthuis, D. N. Granger, M. I. Townsley and A. E. Taylor, *Circulation Res.* **57**, 599 (1985).
36. D. A. Peterson, B. Kelly and J. M. Gerrard, *Biochem. biophys. Res. Commun.* **137**, 76 (1986).
37. B. Hammond and M. L. Hess, *J. Am. Coll. Cardiol.* **6**, 215 (1985).
38. F. Z. Meerson, V. E. Kagan, V. P. Kozlov, L. M. Belkina and Y. V. Arkhipenko, *Basic Res. Cardiol.* **77**, 465 (1982).
39. H. L. Hess, N. H. Manson and K. E. Okabe, *Can. J. Physiol. Pharmac.* **60**, 1382 (1982).
40. I. Fridovich, *Science* **201**, 875 (1978).
41. A. Petkau and N. S. Dhalla, *Can. J. Physiol. Pharmac.* **60**, 1327 (1982).
42. M. Shlafer, P. F. Kane, V. Y. Wiggins and M. M. Kirsh, *Circulation* (Suppl. 1), 185 (1982).
43. B. Woodward and M. N. Zakaria, *J. molec. cell. Cardiol.* **17**, 485 (1985).
44. P. S. Rao, M. V. Cohen and H. S. Mueller, *J. molec. cell. Cardiol.* **15**, 713 (1983).
45. H. L. Hess, E. Okabe and H. A. Kontos, *J. molec. cell. Cardiol.* **13**, 766 (1981).
46. S. R. Jolly, W. J. Kane, M. B. Bailie, G. D. Abrams and B. R. Luchessi, *Circulation Res.* **54**, 277 (1984).
47. S. W. Werns, M. J. Shea, E. M. Driscoll, C. Cohen, G. D. Abrams, B. Pitt and B. R. Luchessi, *Circulation Res.* **56**, 895 (1985).
48. R. S. Roy and J. M. McCord, in *Oxyradicals and Their Scavenger Systems. Cellular and Medical Aspects* (Eds R. A. Greenwald and G. Cohen), p. 145. Elsevier, New York (1983).
49. R. Roy, D. Chambers, J. McCord and J. Downey, *Fedn Proc.* **43**, 323 (1984).
50. M. B. Hallett, A. Shandall and H. L. Young, *Biochem. Pharmac.* **34**, 1757 (1985).
51. M. Bednar, B. Smith, A. Pinto and K. M. Mullane, *Circulation Res.* **57**, 131 (1985).
52. H. P. Jones, M. B. Grisham, S. K. Bose, V. A. Shannon, A. Schott and J. M. McCord, *Biochem. Pharmac.* **34**, 3673 (1985).
53. L. S. Vartanyan and S. M. Gurevich, *Acta biophys.* **113**, 95 (1986).