Allopurinol and amlodipine improve coronary vasodilatation after myocardial ischaemia and reperfusion in anaesthetized dogs

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1 We have assessed the effect of allopurinol, amlodipine and propranolol pretreatment on both endothelium-dependent and endothelium-independent coronary vasodilatation *in vivo*, by comparing pre-ischaemic responses with those measured after 60 min of coronary artery occlusion and 30 min of reperfusion in anaesthetized dogs.

2 In 15 untreated dogs ischaemia and reperfusion attenuated the increases in coronary blood flow produced by either acetylcholine ($0.01\text{--}0.05\text{ }\mu\text{g kg}^{-1}$, i.a.) or glyceryl trinitrate ($0.05\text{--}0.2\text{ }\mu\text{g kg}^{-1}$, i.a.), to an average of $39 \pm 4\%$ and $42 \pm 5\%$ of the pre-ischaemic control response, respectively (both $P < 0.05$).

3 In 5 dogs treated with allopurinol (25 mg kg^{-1} , orally, 24 h previously, plus 50 mg kg^{-1} , i.v., 5 min before occlusion), the increases in coronary blood flow after ischaemia and reperfusion (acetylcholine: $78 \pm 12\%$, glyceryl trinitrate: $60 \pm 3\%$ of pre-ischaemic response) were significantly larger than post-ischaemic responses in untreated dogs (both $P < 0.05$).

4 Similarly, amlodipine treatment ($3\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$, i.v., starting 90 min before occlusion) in 5 dogs improved post-ischaemic increases in blood flow (acetylcholine: 58.5% , glyceryl trinitrate: $66 \pm 6\%$ of pre-ischaemic response, significantly greater than post-ischaemic responses in untreated dogs, $P < 0.05$).

5 In contrast, in a further 6 dogs pretreated with propranolol (1 mg kg^{-1} , i.v., 30 min before occlusion, plus $0.5\text{ mg kg}^{-1}\text{ h}^{-1}$, i.v.), blood flow responses after ischaemia and reperfusion were not different from post-ischaemic responses in untreated dogs (acetylcholine: $46 \pm 6\%$, glyceryl trinitrate: $46 \pm 6\%$ of pre-ischaemic response).

6 These results suggest that allopurinol and amlodipine protect against the post-ischaemic impairment of endothelium-dependent and endothelium-independent coronary vasodilatation *in vivo* by mechanisms additional to endothelial protection.

Keywords: Acetylcholine; allopurinol; amlodipine; coronary artery; endothelium; ischaemia; glyceryl trinitrate; propranolol; reperfusion; vasodilatation

Introduction

Besides regional myocardial infarction and reduced cardiac function, an acute period of coronary artery occlusion and reperfusion causes non-specific attenuation of dilator responses of the large coronary artery (Sobey *et al.*, 1990) and coronary resistance vessels *in vivo* (Nichols *et al.*, 1988; Mehta *et al.*, 1989b,c; Sobey *et al.*, 1990; Vanhaecke *et al.*, 1990). Endothelium-dependent relaxation of the isolated large coronary artery is also impaired, but in contrast to the *in vivo* findings, endothelium-independent relaxation *in vitro* is normal (Ku, 1982; VanBenthuyzen *et al.*, 1987; Mehta *et al.*, 1989a; Sobey *et al.*, 1990; Lefer *et al.*, 1991). These observations indicate that whilst coronary artery endothelial dysfunction is one consequence of ischaemia and reperfusion, other mechanisms besides vascular smooth muscle damage are involved in the non-specific attenuation of coronary dilatation *in vivo* after ischaemia and reperfusion. We have recently reported that administration of amlodipine (a dihydropyridine-type calcium entry inhibitor), allopurinol (a xanthine oxidase inhibitor) or propranolol (a β -adrenoceptor antagonist) *in vivo* prevents the attenuation by ischaemia and reperfusion of endothelium-dependent relaxation *in vitro* (Sobey *et al.*, 1992). These data suggested that processes previously considered to contribute to the post-ischaemic myocardial damage such as excessive levels of intracellular calcium (Kloner & Priskylenk, 1990), generation of cytotoxic oxygen-derived free radicals (Jolly *et al.*, 1984; Ambrosio *et al.*, 1986; Werns *et al.*, 1986; 1988; Chambers *et al.*, 1987; Tamura *et al.*,

1988) and accelerated myocardial energy consumption (Nayler *et al.*, 1980), may also be involved in the impaired endothelial function caused by ischaemia and reperfusion. In the present study we have investigated the protective effects of these agents on both endothelium-dependent and endothelium-independent coronary dilator responses *in vivo* following 60 min of coronary artery occlusion and 30 min of reperfusion.

Methods

Experimental preparation

Thirty-one mongrel dogs of either sex (10–33 kg) were sedated with an intramuscular injection of xylazine ($0.1\text{--}0.5\text{ mg kg}^{-1}$; Bayer, Australia), and anaesthetized by intravenous injection of sodium thiopentone ($25\text{--}30\text{ mg kg}^{-1}$; May & Baker, Australia), followed by α -chloralose (70 mg kg^{-1} ; BDH Chemicals, England) which was supplemented as necessary. The dogs were ventilated artificially with room air plus additional oxygen as necessary to maintain arterial blood gas and pH levels within the physiological range (P_{O_2} : $85\text{--}110\text{ mmHg}$; P_{CO_2} : $30\text{--}35\text{ mmHg}$; pH: $7.35\text{--}7.45$). Systemic arterial blood pressure was measured by inserting a catheter into the right femoral artery, and connecting it to a Druck pressure transducer. Heart rate was monitored with a cardiotelemetry coupler which was triggered by the electrocardiogram (ECG). A left thoracotomy was performed and the heart was suspended in a pericardial cradle. A proximal segment

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(approximately 1 cm in length) of the left circumflex coronary artery was dissected clear of connective tissue and inserted securely into an electromagnetic flow probe (2.0–2.5 mm internal diameter) connected to a flowmeter (Gould Statham, U.S.A.) for coronary blood flow measurement. The 5-mm tip of a coronary arterial catheter, consisting of a 23-gauge hypodermic needle bent at 120° and joined to 30 cm of polyethylene tubing, was inserted into the artery distal to the flow probe. Blood flow in the left circumflex coronary artery was therefore maintained at all times. Intracoronary injections were administered as a bolus of 0.5 ml or less.

Experimental protocol

The effects of ischaemia and reperfusion on coronary dilatation were studied in four groups of dogs: (1) Dogs receiving no pretreatment (untreated, $n = 15$); (2) Dogs pretreated with amlodipine ($3 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v., starting 90 min before arterial occlusion and continuing throughout the period of ischaemia and reperfusion; $n = 5$); (3) Dogs pretreated with allopurinol (25 mg kg^{-1} , orally 24 h previously, plus 50 mg kg^{-1} , i.v., administered 5 min prior to arterial occlusion; $n = 5$); and (4) Dogs pretreated with propranolol (1 mg kg^{-1} , i.v., 30 min prior to arterial occlusion, and supplemented with $0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$ i.v.; $n = 6$). Each dog was included in only one experimental group. In all dogs, left circumflex coronary artery blood flow, systemic arterial blood pressure and heart rate were recorded simultaneously on a Grass model 7D Polygraph. Total coronary vascular resistance was calculated as the quotient of arterial pressure and coronary blood flow. In Groups 1 (in 7 of 15 dogs), 2 and 3 left ventricular pressure was measured with a pressure transducer-tipped catheter (Miller) passed through the left atrium into the left ventricle. Peak positive $LV \text{ dP/dt}$, an index of ventricular contractility, was obtained by electronic differentiation of the left ventricular pressure pulse.

A stabilization period of approximately 20 min was allowed following surgery. In all dogs, control coronary vasodilator responses to acetylcholine (0.01 , 0.02 and $0.05 \mu\text{g kg}^{-1}$) and glyceryl trinitrate (0.05 , 0.1 and $0.2 \mu\text{g kg}^{-1}$) were obtained by injection directly into the left circumflex coronary artery. In pretreated dogs these control dilator responses were obtained in the presence of amlodipine (Group 2) or propranolol (Group 4), or in dogs that had received allopurinol 24 h previously (Group 3). Group 3 dogs were then given the second dose of allopurinol 5 min before coronary occlusion. All variables were allowed to return to baseline levels for at least 5 min between injections.

Five min before occlusion of the left circumflex coronary artery, lignocaine hydrochloride (50 mg , i.v.; Astra Pharmaceuticals, Australia) was administered to reduce arrhythmias. Total occlusion of the left circumflex coronary artery, proximal to the flow probe and distal to the first main branch, was performed with a small bulldog clip. After 60 min of arterial occlusion the clip was removed, allowing reperfusion of the ischaemic myocardium. Left circumflex coronary artery blood flow was restricted to the pre-occlusion level for approximately 10 min by partially occluding the artery with a 10 cm length of cotton tape placed around the artery. This procedure limits reperfusion hyperaemia, thus reducing the severity of reperfusion arrhythmias and the incidence of haemorrhagic myocardial infarction (Lucchesi *et al.*, 1976). Recordings of the ECG (leads I, II and III) were made periodically in each experiment. After 30 min of reperfusion, vasodilator injections were repeated. The effect of coronary artery occlusion and reperfusion on baseline levels of all variables was studied by comparing resting levels recorded: (i) 10 min before coronary artery occlusion, (ii) at the end of the 60 min occlusion period, (iii) after 30 min of reperfusion, and (iv) at the end of the experiment (106 ± 7 min of reperfusion in Group 1; 75 ± 8 min in Group 2; 103 ± 6 min in Group 3; and 111 ± 14 min in Group 4).

Drugs

Acetylcholine perchlorate (BDH Chemicals, Poole, Dorset) and ($\pm$)-propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) were dissolved and diluted in 0.9% saline. Glyceryl trinitrate (GTN, Tridil, Boots Co., Australia) was diluted in 0.9% saline. Amlodipine maleate (Pfizer, Sandwich, Kent) was dissolved and diluted in water for injection. Allopurinol (Sigma) was dissolved in 50 ml 0.9% saline (high pH with 2 M NaOH).

Calculations and statistics

All data presented are the mean $\pm$ s.e. mean of n experiments. A one-way analysis of variance and Neuman-Keul's test for multiple comparisons were used to analyse the effect of drug treatment and ischaemia/reperfusion on baseline levels. Dose-response curves generated by acetylcholine and glyceryl trinitrate before and after ischaemia/reperfusion were compared using a two-way analysis of variance and Neuman-Keul's test for multiple comparisons.

To evaluate the protection against ischaemia-induced impairment of coronary vasodilatation by each pharmacological agent, post-ischaemic dilator responses in Groups 2–4 were compared with those in Group 1. For this analysis, post-ischaemic increases in coronary blood flow produced by acetylcholine and glyceryl trinitrate were expressed as a percentage of the pre-ischaemic response, and an average value for each dilator was then calculated in each experiment. The mean of these post-ischaemic values in each of Groups 2–4 was then compared with the Group 1 value by a one-way analysis of variance and a planned comparison test. A P value of less than 0.05 was considered to be statistically significant in each analysis.

Results

Effects of vasodilators on cardiovascular variables before coronary occlusion

Intra-coronary injection of either acetylcholine (0.01 – $0.05 \mu\text{g kg}^{-1}$) or glyceryl trinitrate (0.05 – $0.2 \mu\text{g kg}^{-1}$) produced a dose-dependent increase in coronary blood flow in all groups (Figures 1–4). There was no change in arterial pressure, heart rate or $LV \text{ dP/dt}$ produced by these doses of the dilators (data not shown).

Effects of pharmacological pretreatments on baseline cardiovascular variables before coronary occlusion

Amlodipine treatment resulted in a reduced systemic arterial pressure compared with untreated dogs (Table 1, $P < 0.05$, one-way analysis of variance). Allopurinol administration caused an elevation of heart rate for approximately 30 min (Table 1, $P < 0.05$, one-way analysis of variance). No other variable was significantly different from the control group following pretreatment (Table 1). However, arterial pressure and heart rate were significantly reduced and coronary dilator responses to isoprenaline ($0.01 \mu\text{g kg}^{-1}$, i.a.) were abolished in Group 4 dogs after administration of propranolol ($P < 0.01$, paired t test, data not shown).

Effect of coronary occlusion and reperfusion on baseline variables

Coronary artery occlusion and reperfusion tended to reduce arterial pressure, but this effect was statistically significant only in propranolol-treated dogs at the end of the occlusion period (Table 1, $P < 0.05$, one-way analysis of variance). Coronary blood flow in Groups 3 and 4 remained significantly reduced after reperfusion (Table 1, $P < 0.05$, one-way analysis of variance). $LV \text{ dP/dt}$ was reduced after coronary

Table 1 Effect of left circumflex coronary artery occlusion and reperfusion on baseline variables

Group	10 min Pre-occlusion	60 min Occlusion	30 min Reperfusion	End Expt.
a AP (mmHg)				
1: Untreated	114 ± 5	107 ± 4	104 ± 4	108 ± 4
2: Amlodipine	94 ± 7*	91 ± 8	92 ± 8	87 ± 7
3: Allopurinol	101 ± 6	98 ± 7	106 ± 6	106 ± 6
4: Propranolol	120 ± 3	99 ± 8**	104 ± 6	108 ± 7
b CBF (ml min⁻¹)				
1: Untreated	30 ± 4	0**	25 ± 4	27 ± 4
2: Amlodipine	38 ± 7	0**	32 ± 2	36 ± 5
3: Allopurinol	36 ± 7	0**	18 ± 4**	19 ± 4**
4: Propranolol	33 ± 4	0**	20 ± 2**	22 ± 2**
c HR (b min⁻¹)				
1: Untreated	134 ± 8	135 ± 8	131 ± 8	131 ± 7
2: Amlodipine	121 ± 11	124 ± 13	127 ± 15	128 ± 16
3: Allopurinol	167 ± 7*	131 ± 7**	121 ± 5**	126 ± 7**
4: Propranolol	121 ± 6	117 ± 5	114 ± 6	111 ± 6
d CVR (mmHg min ml⁻¹)				
1: Untreated	4.9 ± 0.8	—	5.9 ± 1.0	5.5 ± 1.0
2: Amlodipine	2.8 ± 0.5	—	2.9 ± 0.3	2.5 ± 0.3
3: Allopurinol	3.4 ± 0.7	—	8.8 ± 3.6	7.6 ± 2.7
4: Propranolol	4.0 ± 0.5	—	5.4 ± 0.6	5.3 ± 0.7

AP: arterial pressure; CBF: coronary blood flow; HR: heart rate; CVR: coronary vascular resistance.

*Significantly different from Group 1; **Significantly different from 10 min pre-occlusion value; $P < 0.05$, one-way analysis of variance.

occlusion and reperfusion only in untreated dogs, indicating that both amlodipine and allopurinol prevented the reduction in cardiac contractility caused by ischaemia and reperfusion ($P < 0.05$, one-way analysis of variance, data not shown; $LV\ dP/dt$ was not measured in Group 4). No other variable was significantly altered by coronary occlusion and reperfusion. Alterations in the ECG indicative of myocardial ischaemia were observed following coronary artery occlusion in all dogs (data not shown).

Effect of coronary occlusion and reperfusion on coronary dilator responses

Group 1: Untreated dogs Increases in coronary blood flow produced by both acetylcholine and glyceryl trinitrate were significantly reduced after 60 min of coronary occlusion and 30 min of reperfusion (Figure 1; $P < 0.05$, two-way analysis of variance). Overall, post-ischaemic increases in coronary blood flow produced by acetylcholine and glyceryl trinitrate were reduced to $39 \pm 4\%$ and $42 \pm 5\%$ of the pre-ischaemic control response, respectively (Figure 5; both significantly less than control response, $P < 0.05$, one-way analysis of variance).

Group 2: Amlodipine-treated dogs In dogs pre-treated with amlodipine, post-ischaemic increases in coronary blood flow produced by glyceryl trinitrate were not significantly different from control responses (Figure 2). Increases in coronary blood flow produced by acetylcholine after ischaemia and reperfusion were significantly reduced in comparison to control (Figure 2; $P < 0.05$, two-way analysis of variance). However, post-ischaemic dilator responses to both acetylcholine ($58 \pm 5\%$ of control) and glyceryl trinitrate ($66 \pm 6\%$ of control) in amlodipine-treated dogs were significantly greater than those measured in Group 1 dogs (Figure 5; both are $P < 0.05$ compared with Group 1, one-way analysis of variance). Thus, amlodipine partially protected against impairment of both endothelium-dependent and endothelium-independent coronary vasodilatation following ischaemia and reperfusion.

Group 3: Allopurinol-treated dogs Allopurinol pretreatment prevented a significant reduction of acetylcholine-induced

Figure 1 Dose-response effects on coronary blood flow (CBF) produced by intra-coronary injection of acetylcholine (ACh, a) and glyceryl trinitrate (GTN, b) in untreated dogs (Group 1). Control responses for either dilator (●) are significantly greater than those produced after 60 min of coronary artery occlusion and 30 min of reperfusion (□). * $P < 0.05$, two-way analysis of variance.

Figure 2 Dose-response effects on coronary blood flow (CBF) produced by intra-coronary injection of acetylcholine (ACh, a) and glyceryl trinitrate (GTN, b) in amlodipine-treated dogs (Group 2). Control responses (●) for ACh are significantly greater than those produced after 60 min of coronary artery occlusion and 30 min of reperfusion (□). * $P < 0.05$, two-way analysis of variance; there is no significant difference between control and post-ischaemic responses to GTN.

Figure 3 Dose-response effects on coronary blood flow (CBF) produced by intra-coronary injection of acetylcholine (ACh, a) and glyceryl trinitrate (GTN, b) in allopurinol-treated dogs (Group 3). There is no significant difference between control responses (●) to ACh and those obtained after 60 min of coronary artery occlusion and 30 min of reperfusion (□). Control responses to GTN are significantly greater than post-ischaemic responses: * $P < 0.05$, two-way analysis of variance.

Figure 5 Increases in coronary blood flow (CBF) after 60 min of coronary artery occlusion and 30 min of reperfusion expressed as a percentage of the control response produced before ischaemia (Pre-isch). Post-ischaemic dilator responses to both acetylcholine (a) and glyceryl trinitrate (b) are significantly greater in dogs treated with amlodipine (Aml) and allopurinol (All) than in untreated dogs (Unt) or dogs treated with propranolol (Pro). * $P < 0.05$, one-way analysis of variance.

Figure 4 Dose-response effects on coronary blood flow (CBF) produced by intra-coronary injection of acetylcholine (ACh, a) and glyceryl trinitrate (GTN, b) in propranolol-treated dogs (Group 4). Control responses for either dilator (●) are significantly greater than those produced after 60 min of coronary artery occlusion and 30 min of reperfusion (□). * $P < 0.05$, two-way analysis of variance.

dilator responses after ischaemia and reperfusion (Figure 3). Glyceryl trinitrate-induced responses were significantly reduced in comparison to control (Figure 3; $P < 0.05$, two-way analysis of variance), but both acetylcholine- and glyceryl trinitrate-induced responses after ischaemia and reperfusion ($78 \pm 12\%$ and $60 \pm 3\%$, respectively) were significantly improved by allopurinol treatment (Figure 5; both $P < 0.05$ compared with Group 1, one-way analysis of variance).

Group 4: Propranolol-treated dogs Increases in coronary blood flow produced by both acetylcholine and glyceryl trinitrate were significantly reduced after 60 min of coronary occlusion and 30 min of reperfusion (Figure 4; both $P < 0.05$). Mean post-ischaemic increases in coronary blood flow produced by acetylcholine and glyceryl trinitrate were both $46 \pm 6\%$ of the respective pre-ischaemic control responses (Figure 5; both values not different from Group 1). Thus propranolol did not protect against ischaemia-induced impairment of coronary vasodilatation.

Discussion

This study has confirmed previous reports (Mehta *et al.*, 1989b; Sobey *et al.*, 1990) that increases in coronary blood flow produced by either acetylcholine (an endothelium-dependent dilator) or glyceryl trinitrate (an endothelium-independent dilator) are markedly reduced after an acute period

of ischaemia and reperfusion in anaesthetized dogs. In this model, large coronary artery dilatation by both agents *in vivo* is also impaired after ischaemia and reperfusion (Sobey *et al.*, 1990). However, we (Sobey *et al.*, 1990; 1992) and others (Ku, 1982; Vanbenthuyzen *et al.*, 1987; Mehta *et al.*, 1989a; Lefer *et al.*, 1991) have shown that in isolated coronary arteries previously exposed to ischaemia and reperfusion, endothelium-independent relaxation remains intact. Thus, *in vitro* findings do not necessarily reflect the post-ischaemic coronary vasodilator capacity *in vivo*, highlighting the importance of examining the effects of ischaemia on vascular function in the intact circulation. We recently reported that *in vivo* administration of amlodipine (a calcium entry inhibitor), allopurinol (a xanthine oxidase inhibitor) or propranolol (a β -adrenoreceptor antagonist) significantly protects endothelial function in ischaemic and reperfused large coronary arteries *in vitro* (Sobey *et al.*, 1992). In the present study we have examined whether these pharmacological agents can also prevent the non-specific attenuation by ischaemia and reperfusion of coronary resistance vessel dilatation *in vivo*.

The present findings indicate that amlodipine and allopurinol partially prevent attenuation of both endothelium dependent and endothelium-independent increases in coronary blood flow after ischaemia and reperfusion. Both of these agents also prevented a post-ischaemic impairment in cardiac contractility, consistent with their ability to reduce myocardial infarct size (Werns *et al.*, 1986; Chambers *et al.*, 1987; Hoff *et al.*, 1989). In contrast, protection of endothelial function by propranolol (Sobey *et al.*, 1992) is clearly not sufficient to prevent the attenuation of coronary resistance vessel dilatation *in vivo* after ischaemia and reperfusion. Therefore, in addition to endothelial damage, there must be processes occurring *in vivo* that cause non-specific impairment of post-ischaemic coronary dilator mechanisms.

The protective effects of amlodipine and allopurinol are further evidence that elevated intracellular calcium levels and superoxide anion production, respectively, may be involved in the impairment of vasodilator function after ischaemia and reperfusion. Inhibition of calcium entry by amlodipine during ischaemia and reperfusion may be beneficial in a number of cell types. For example, activation of leukocytes and platelets would be inhibited, and the reduced contractility of cardiac and vascular smooth muscle would limit oxygen demands and hence the hypoxic damage to these cells. As ischaemia and reperfusion does not cause vascular smooth muscle damage in this model (Vanbenthuyzen *et al.*, 1987; Mehta *et al.*, 1989a; Sobey *et al.*, 1990; 1992), the protection by allopurinol and amlodipine is probably not mediated by an action on that cell type.

Infiltration of circulating leukocytes into microvessels of the ischaemic myocardium could be an important contributing factor in the reduction of post-ischaemic dilator responses. This event results in the physical obstruction, or 'plugging', of small coronary vessels, and is associated with a gradual reduction of post-ischaemic coronary blood flow known as the 'no-reflow' phenomenon (Kloner *et al.*, 1974; Engler, 1987). A reduced coronary vasodilator reserve then results from the consequent dilatation of non-occluded microvessels to maintain resting coronary blood flow. In addition to these physical effects, the accumulation of activated leukocytes in ischaemic and reperfused coronary arteries (Mullane *et al.*, 1984; Sobey *et al.*, 1990) and myocardium (Romson *et al.*, 1983; Engler *et al.*, 1983; Mullane *et al.*, 1984; Nichols *et al.*, 1988; Sobey *et al.*, 1990; Sheridan *et al.*, 1991; Lefer *et al.*, 1991) is associated with tissue damage

that is thought to be mediated by oxygen-derived free radicals. At least part of the protection by amlodipine and allopurinol could therefore involve an inhibitory effect on leukocyte infiltration and/or function. Such an action by amlodipine is possible, since two other dihydropyridine calcium antagonists, nifedipine and nisoldipine, inhibit the generation of several human neutrophil products, including superoxide anion (Jouvin-Marche *et al.*, 1983; Irita *et al.*, 1986). This effect of the calcium antagonists may be independent of their inhibition of calcium influx, for nisoldipine inhibited NADPH oxidase and thus superoxide generation by neutrophils in the absence of extracellular calcium (Irita *et al.*, 1986). Inhibition of leukocyte functions by amlodipine might ultimately produce a number of protective actions resembling those of superoxide dismutase, a well known scavenger of superoxide anions, which inhibits both neutrophil accumulation and the reduction of post-ischaemic coronary vasodilator reserve in ischaemic myocardium of dogs (Mehta *et al.*, 1989c). In combination with the hydrogen peroxide scavenger catalase, superoxide dismutase also improves regional coronary blood flow and decreases microvascular injury after ischaemia and reperfusion in dogs (Przyklenk & Kloner, 1989). Whilst allopurinol inhibits superoxide anion generation by xanthine oxidase, an enzyme localized in the vascular endothelium (Ferrari *et al.*, 1990), it does not inhibit superoxide production, chemotaxis or degranulation by human neutrophils (Jones *et al.*, 1985; Capecchi *et al.*, 1988). Therefore, a direct action by allopurinol on leukocytes in this study is unlikely.

Ischaemia-induced vasoconstriction (Sobey *et al.*, 1990; Chu *et al.*, 1990) could be partly due to a reduction in basal release of the vasodilators nitric oxide and prostacyclin from damaged endothelial cells. However, this process probably did not contribute to the reduction in coronary vasodilatation, since endothelium function *in vitro* (Sobey *et al.*, 1992), but not vasodilatation *in vivo*, was normal in coronary arteries of propranolol-treated dogs. The reason for the sustained reduction in post-ischaemic coronary blood flow in allopurinol- and propranolol-treated dogs only is unclear, but this is unlikely to have influenced the dilator responses to acetylcholine and glyceryl trinitrate because allopurinol, but not propranolol, protected against the impairment of dilatation after ischaemia.

In conclusion, the non-specific attenuation of coronary resistance vessel dilatation that occurs *in vivo* following ischaemia and reperfusion can be inhibited by administration of amlodipine or allopurinol, but not propranolol. These results support the involvement of elevated intracellular calcium levels and superoxide anion generation in the mechanism of post-ischaemic vasodilator dysfunction. Since all three of these agents prevent post-ischaemic endothelial dysfunction (Sobey *et al.*, 1992), the maintenance of vasodilator reserve by amlodipine and allopurinol probably involves additional mechanisms. An inhibitory action of these drugs on leukocyte infiltration and activation is one such possibility that warrants further investigation. Recently reported evidence that nifedipine reduces the incidence of myocardial infarction following coronary bypass surgery (Seitelberger *et al.*, 1991) further emphasises that such protective actions by amlodipine, allopurinol or chemically related pharmacological agents may be of potential clinical benefit.

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