Vasodilator pre-treatment of human radial arteries

Comparison of effects of phenoxybenzamine vs papaverine on norepinephrine-induced contraction in vitro

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Aims The radial artery, increasingly used for coronary artery bypass grafting (CABG), has a potential for spasm which may increase peri-operative risk. Increased alpha-adrenoceptor activation is a key candidate for the spasm. We studied the effects of vasoconstriction in a radial artery, which had undergone brief exposure to the alpha-adrenoceptor antagonist phenoxybenzamine vs the opioid derivative papaverine.

Methods and Results Using standard classical organ bath techniques, concentration responses were obtained to norepinephrine in segments of radial artery from 12 CABG patients pre- and post-incubation for 20 min in either phenoxybenzamine 10−6 M or papaverine 3×10−3 M. Responses were reassessed 2, 4 and 18 h after washout of phenoxybenzamine and 2, 4, 8 and 18 h after washout of papaverine. There was concentration-dependent constriction to norepinephrine (maximum response 0·89 ± 0·20 (SEM) g.mm−1, n=6). Constriction to norepinephrine was abolished immediately after incubation in phenoxybenzamine and remained completely inhibited for at least 18 h (P<0·0001 ANOVA phenoxybenzamine pre-treated vs controls). Most of the inhibition of concentration-dependent constriction to norepinephrine following pre-treatment with papaverine was lost 8 h later.

Conclusion Radial artery vasoconstriction induced by a clinically relevant agonist, norepinephrine, may be prevented for at least 18 h by pre-incubation in phenoxybenzamine, in contrast to the brief inhibition achieved by pre-treatment with papaverine. Adding phenoxybenzamine to radial artery graft bathing solution may improve early outcome following CABG.


Key Words: Coronary disease, norepinephrine, alpha-adrenergic receptors, surgery, vasoconstriction.

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Introduction

Increasing use of arterial grafts as conduits for coronary artery bypass surgery has led to recognition of a short-term complication characterized by low flow in the coronary vasculature distal to the graft and poor myocardial perfusion[1–4]. To treat the syndrome, systemic administration of inotropes such as adrenaline may be used to maintain cardiac output. This may further compromise myocardial perfusion and may thus precipitate heart failure or myocardial infarction. This low-flow syndrome usually occurs during the initial 48 h after surgery, has been reported with internal mammary artery grafts as well as radial artery grafts[1–2] and is common. Acar et al. reported thread-like vessels on angiography in 4% of artery grafts, suggesting marked vasoconstriction[3]. Other groups, using less stringent clinical criteria, have suggested that this hypoperfusion syndrome may occur in up to 10% of cases[4].

A wide range of different approaches are in current use to try to limit the development of this clinically important syndrome, including in vitro pre-incubation of the graft with vasodilators such as the phosphodiesterase G inhibitor papaverine.

An important candidate mediator of the syndrome is increased alpha-adrenoceptor pathway activation. Although pre-incubation with papaverine is used in several major centres in Europe and in the U.S.A., it is short-acting and effects of pre-incubation of grafts with papaverine on alpha-1 adrenoceptor activation are not
clear. The expected constriction was prevented when epinephrine was added to radial arteries immediately after they had been incubated for 60 min with a high concentration (1 mg.ml⁻¹) of the alpha-adrenoceptor blocker phenoxybenzamine⁶⁹. A 1-h incubation period is not clinically practical for coronary bypass surgery. We studied the effects of a brief 20-min pre-incubation of radial arteries with a lower, clinically appropriate concentration of phenoxybenzamine on catecholamine-induced vasoconstriction. We compared the efficacy and duration of these effects to those of pre-incubation with papaverine.

**Methods**

We studied 12 patients undergoing elective coronary artery bypass. Eleven were men, mean age 64 (range 53–74) years and three had diabetes mellitus. As expected in these patients with multiple cardiovascular risk factors and established coronary artery disease, treatment included statins, aspirin, nitrates, ACE inhibitors, calcium channel blockers, beta-blockers, diuretics and antidiabetic drugs. Direct effects of previous oral drug treatment were minimized by careful washing of the segments and by constant perfusion of vascular rings throughout the experiments (see below). The study was approved by the local hospital ethics committee.

We studied segments of radial arteries harvested at the time of bypass. Radial arteries were harvested from the mid-arm. Vascular rings were obtained for in vitro studies randomly from proximal and distal ends of the harvested artery. Within each in vitro experiment, rings from the same section of radial artery were used for active treatment and as controls. The above protocol minimized any possible confounding effects of regional differences in receptor densities for out measurements. Ex vivo, the artery segments were not exposed to any exogenous vasodilators prior to collection. The arteries were removed with adjacent veins and surrounding subcutaneous fat and were placed in ice-cold physiological saline solution. The vessels were dissected free of adventitia in ice-cold physiological saline solution under magnification within 60 min of harvesting. Five-millimetre ring segments of vessel were cut and mounted on a classical organ bath. Two to four segments were taken from each vessel.

The organ bath techniques were as previously reported⁶⁶ and are described here in brief. Arterial segments were mounted on two stainless steel stirrups placed through the lumen of the vessels. The upper stirrup was attached to a force transducer. The tension to which the vessels were subjected was increased manually in increments of 1 g across the range from 1 g to 7 g over 30 min to achieve the maximum active tension for the minimum resting tension. From previous length tension experiments⁶⁸ this allows approximation to a circumference of 90% of the internal circumference of the artery at a transmural pressure of 100 mmHg. Rings were then allowed to equilibrate for a further 60 min at this tension. During all incubations of greater than 20 min, vessels were washed at a rate of 1 ml.min⁻¹ with oxygenated physiological saline solution at 37 °C. Following equilibration, arterial segments were contracted with psychological saline solution containing 124 mm potassium to obtain maximum smooth muscle contraction. The contractions were repeated three times or until the tension achieved in two consecutive contractions was within 10%. The vessels were washed and allowed to equilibrate after contraction. For all vascular rings, constrictor concentration response curves were then obtained by adding increasing concentrations of norepinephrine to the organ bath in half-log molar incremental doses at 2-min intervals to give final concentrations from 10⁻⁸ M (2 ng.ml⁻¹) to 10⁻³ M (2 μg.ml⁻¹).

The vessels were then washed and pre-constricted with 10⁻⁶ M norepinephrine. This concentration of norepinephrine in these studies gave a contraction that was 80% of maximal. Once the contraction had reached a stable plateau, in order to assess the endothelial integrity of the vessels, increasing concentrations of acetylcholine were added to the organ bath in incremental doses to give final concentrations of 10⁻⁸ M to 10⁻³ M. The vessels were then washed and incubated for 20 min in the presence of either psychological saline solution, papaverine (final concentration of 3 x 10⁻⁵ M [1 mg.ml⁻¹]) or phenoxybenzamine (final concentration 10⁻⁶ M [34 μg.ml⁻¹]). At the end of the incubation a further norepinephrine concentration curve was constructed. The vessels were then washed and norepinephrine concentration responses were repeated at 2, 4 and 18 h post-washout of the pre-treatment agent in the phenoxybenzamine treated vessels and after 2, 4, 8, 18 h in the papaverine-treated vessels. To assess contractile viability of the arterial segments at the end of the 18 h of incubation, all vessels were constricted with 124 mm potassium solution and the thromboxane A₂ agonist U46619 (at a final concentration of 10⁻⁶ M).

**Solutions and drugs**

The physiological saline solution was buffered to pH 7.4, maintained at 37 °C and bubbled with 5% CO₂, 95% O₂ and consisted of mm: NaCl 119, NaHCO₃ 25, d-glucose 5-5, KH₂PO₄ 1·18, MgSO₄ 7H₂O 1·17, KCl 4·7, CaCl₂ 2·5, ethylenediaminetetraacetic acid disodium salt (EDTA) 0·026 (AnalaR quality; BDH Chemicals Ltd, Poole, U.K.). The high potassium physiological saline solution used as a constricting agent consisted of (mm): KCl 124·4, NaHCO₃ 25, d-glucose 5·5, KH₂PO₄ 1·18, MgSO₄ 7H₂O 1·17, CaCl₂ 2·5, ethylenediaminetetraacetic acid disodium salt (EDTA) 0·026. The drugs used were noradrenaline (arterenol hydrochloride), acetylcholine chloride and thromboxane U46619 (all from Sigma Chemical Co., St Louis, MO, U.S.A. and Sigma-Aldrich Chemie GmbH, Steinheim, Germany), papaverine (papaverine hydrochloride, BP
norepinephrine was 21/p5 control vessels in respective experiments. with papaverine or phenoxybenzamine compared with phrine between the vessels subsequently incubated. NOVA were used when comparing individual data. Least signifi cant. Least significant difference values derived from the ANOVA were used when comparing individual data points.

**Results**

*Initial contraction to norepinephrine*

Prior to treatment all vessels showed concentration-dependent contraction to increasing incremental half-log molar doses of norepinephrine from 10⁻⁸ to 10⁻³ M. No differences were seen in the sensitivities to norepinephrine between the vessels subsequently incubated with papaverine or phenoxybenzamine compared with control vessels in respective experiments.

*Effects of pre-incubation for 20 min in papaverine*

The addition of 3 × 10⁻³ M papaverine had no effect on the resting tone of the radial artery segments. However at the end of 20 min of incubation in papaverine, the contractile response to norepinephrine was abolished (ANOVA P=0.019 treated vs control vessels: Fig. 1). This complete inhibition of constriction remained for 2 h after wash-out of papaverine. By 4 h after pre-treatment with papaverine, the contraction to 10⁻³ M norepinephrine was 21 ± 9% of control vessels (Fig. 1). By 8 h after pre-incubation with papaverine, there was markedly reduced inhibition of the contractile response to norepinephrine (vs control vessels P=0.319, ANOVA; Fig. 1). At 18 h after pre-treatment with papaverine, there was no residual difference in norepinephrine-induced constriction between pre-treated and control vessels (Fig. 1).

*Effects of pre-incubation for 20 min in phenoxybenzamine*

The addition of 10⁻⁶ M phenoxybenzamine had no effect on the resting tone of the radial artery segments. However, at the end of 20 min of incubation in phenoxybenzamine 10⁻⁶ M, the contractile response to norepinephrine was abolished (treated vs control vessels P=0.001, ANOVA: Fig. 2). Constriction to the entire concentration range of norepinephrine studied was inhibited for at least 18 h after pre-treatment with phenoxybenzamine (pre-treated vs control vessels: P<0.0001, ANOVA; Fig. 2).

*Vessel viability*

**Contractile responses**

The radial artery preparations showed a mean absolute contraction to 124 mM potassium solution of 6.16 ± 0.37 g prior to treatment. There was no statistical difference between maximum contractions seen at the start of the experiments for arteries used for incubation with papaverine or phenoxybenzamine (6.27 ± 0.59 g) vs control arteries (5.69 ± 0.49 g; P=0.09). When vessels were re-studied 18 h after pre-treatment with either phenoxybenzamine, papaverine or control (psychological saline solution) there was no statistical difference in contractile responses versus control vessels within either treatment group on challenge with 124 mM potassium chloride (KCl: pre-treated vessels 5.40 ± 0.50 g vs control 5.40 ± 0.57 g P=0.95) or the thromboxane A₂ agonist U46619 (U46619: pre-treated vessels 5.58 ± 0.67 g versus control 5.79 ± 0.53, P=0.73; Fig. 3).

**Endothelium-dependent relaxation**

The response to the endothelium-dependent relaxing factor acetylcholine was tested prior to treatment and no significant difference was seen between control and treatment groups (phenoxybenzamine treated versus control P=0.70, papaverine treated versus control P=0.53, ANOVA). Maximum relaxation in response to 10⁻⁵ M acetylcholine for papaverine-treated arteries was 56% versus 66% for control arteries and 83% for phenoxybenzamine-treated arteries versus 71% for control arteries. Two vessels in the papaverine group failed to relax by more than 50% of the pre-constricting tension. The exclusion from the analysis of these vessels did not alter the outcome of the experiments.

*Discussion*

The ideal agent for prevention of radial artery spasm has been the subject of several recent investigations because of renewed current interest in this vessel as an alternative...
conduit for coronary bypass surgery. We have shown for the first time that brief pre-treatment with the alpha-adrenergic antagonist phenoxybenzamine prevents contraction for at least 18 h when the human radial artery is challenged with norepinephrine. In contrast, the protective effects of papaverine were relatively short-lived. Our findings could be important in providing an additional new approach to prevention of the hypoperfusion syndrome early after coronary artery bypass surgery, in particular because of the well-recognized increase in circulating levels of catecholamines during and after cardiopulmonary bypass.

Saphenous veins grafts were commonly used as conduits following the introduction of coronary artery

Figure 1  Contraction to half-log molar increases in concentration of norepinephrine in control radial artery and in radial artery after 20 min of incubation in papaverine and 4, 8 and 18 h post-exposure to papaverine (n=6). The upper panel shows control results for radial artery incubated for 4 h in psychological saline solution alone, the lower panel control results for radial artery incubated for 18 h in psychological saline solution alone (*P=0·019 ANOVA for papaverine treated vessels vs control). Each point represents mean ± SEM expressed as a percentage of maximum contraction to 124 mM KCl.
bypass graft (CABG) surgery in the early 1960s. Several groups went on to note increased early and late patency of arteries such as the internal mammary compared with saphenous vein as graft conduits. Cameron et al. reported that 15 years after CABG surgery, two-thirds of their patients receiving a single internal mammary artery remained free of myocardial infarction compared with only half in the saphenous vein group. Mean survival was also better in the arterial conduit patients. A wide range of other arteries are also now in regular use as bypass conduits including the gastro-epiploic, inferior epigastric and radial artery. Initial results with the radial artery in the early 1970s were poor, with a high proportion of early post-operative graft occlusion when assessed angiographically. Since then, better harvesting,

**Figure 2** Contraction to half log molar increases in concentration of norepinephrine in control radial artery and in radial artery after 20 min incubation in phenoxybenzamine and 18 h post-exposure to phenoxybenzamine (n=6). The upper panel shows control results (n=6) for radial artery incubated for 20 min in psychological saline solution alone, the lower panel control results for radial artery incubated for 18 h in psychological saline solution alone (*P=0.001 ANOVA for phenoxybenzamine treated vessels vs control vessels). Each point represents mean ± SEM expressed as a percentage of maximum contraction to 124 mM KCl.
Several empirical strategies have been used to try to prevent this syndrome. In many centres, a solution of papaverine, a phosphodiesterase G inhibitor with some calcium channel blocking actions, is used to pre-treat and distend the conduit artery prior to grafting[19]. Cooper et al. studied pre-treatment of internal mammary artery pedicles in situ with nifedipine, papaverine, glyceryltrinitrate, sodium nitroprusside or saline pre-bypass surgery. Flow increased by 72% with papaverine, by around 200% with nifedipine or glyceryltrinitrate and 250% with sodium nitroprusside. However, only acute relaxant effects on basal tone were studied and the ability of these agents to antagonize exogenous constrictor agonists was not assessed[19]. Recently, nitroglycerin in combination with calcium channel blockers such as verapamil or diltiazem has also been used for pre-incubation or systemic administration[20,21]. He and Yang reported inhibition of constriction to 100 mM potassium solution in radial arteries pre-treated with verapamil and nitroglycerin and studied 24 h later.

However, the ability of these agents to inhibit receptor-mediated constriction was not tested and baseline function in the stored vascular rings was not established. Furthermore these arteries had been stored at 4°C in a refrigerator[20]. Thus the duration of action of these agents may have been artificially prolonged and the possible benefits of pre-treatment overstated, as in some vessels relaxation may have reflected poor basal function. Our studies are more reflective of the clinical setting, in that our pre-treated vascular rings were perfused at 37°C throughout the 18 h of each experiment. In addition, we obtained serial assessment of vascular rings in all control and treated vessels.

Shapira et al. tested approaches to reversing established radial spasm using an in vitro model. They compared relaxant effects of nitroglycerin with diltiazem in radial artery segments constricted with the thromboxane mimic U46619 in vitro. The calcium antagonist diltiazem was ineffective, whereas nitroglycerin increased the radial artery diameter by 22%/23]. This supports the use of nitroglycerin to treat established thromboxane-mediated radial spasm. However He and Yang provide a cautionary note, finding that radial arteries pre-treated with nitroglycerin developed tolerance to the subsequent administration of the drug[22]. Relaxation to the alternative nitric oxide donor sodium nitroprusside was not limited by tolerance[22], however, its clinical use is complicated by the potential accumulation of cyanide in the circulation[23].

The response to calcium channel blockers has been inconsistent. Cable et al.[24], in an important study exploring both conventional pharmacology and gene therapy, reported no significant inhibition of norepinephrine-induced constriction by pre-treatment with diltiazem or verapamil, but found relaxation of norepinephrine-mediated constriction by pre-treatment with the short-acting dihydropryidine calcium antagonist nifedipine. In that study, pre-treatment with either of the nitric oxide donors, isosorbide dinitrate or nitrroglycerin, inhibited constriction to norepinephrine and to KCl. The duration of protection from these constrictor agents by pre-treatment of radial arteries with these
relaxing agents was not assessed. As noted above, a potential limitation of sustained administration of nitricates is tolerance, in addition to the potential haemodynamic side-effects of prolonged nitrate treatment. Cable et al. also demonstrated both expression of eNOS and functional effects in radial artery segments with improved relaxation to KCl and to prostaglandin F$_2$α [24]. Of interest, expression was confirmed after 40 h of incubation, beyond the usual timeframe for the early radial artery vasospasm, but clearly of great potential interest for longer term prophylaxis.

Several lines of evidence support activation of the alpha-adrenoceptor pathway as an important candidate mediator of the hypoperfusion syndrome. Anaesthesia, hypothermia and circulatory arrest on cardiopulmonary bypass result in increases in the circulating levels of epinephrine and norepinephrine. Endogenous levels of catecholamines are around 3-fold increased in association with cardiopulmonary bypass [25] with around 20-fold higher levels resulting from deep hypothermia and circulatory arrest [26]. These raised levels persist for around 24 h. Furthermore, exogenous catecholamines are commonly used peri-operatively for circulatory support after coronary artery bypass grafting. Exogenous inotropes with alpha-1 agonist effects, such as norepinephrine, and higher doses of dopamine may be used clinically in the immediate post-operative period to increase cardiac output. There is also, as noted above, experimental evidence for up-regulation of alpha-1 adrenoceptors in grafted vessels in response to denervation [18].

Our study clearly shows that pre-treatment of radial arteries with papaverine, although initially effective, is short-acting with little residual inhibition of norepinephrine-induced vasoconstriction 8 h after papaverine pre-treatment. In contrast, our study showed that phenoxybenzamine provided long-acting complete inhibition of norepinephrine-induced vasoconstriction for at least 18 h before treatment with the drug. This long duration of inhibition by phenoxybenzamine results from alkylation of alpha-adrenoceptors, leading to a long-lasting non-competitive block. When given systematically, duration of action of this effect in man is in excess of 48 h. Of interest, in view of the probable multifactorial aetiology of the low flow syndrome, is that phenoxybenzamine also inhibits vasoconstriction by other agonists, including that mediated by acetylcholine via muscarinic receptors [27] and serotonin-mediated constriction [28]. Moreover, the renin–angiotensin pathway may also be activated by coronary artery bypass and effects of angiotensin II include stimulation of sympathetic outflow [29] which, through spillover of norepinephrine from nerve endings, could contribute to the increased levels of circulating norepinephrine after CABG. Of possible relevance to the hypoperfusion syndrome is the report that phenoxybenzamine can prevent angina at rest in native coronary arteries [30]. This may result, in part, from inhibition by phenoxybenzamine of alpha-adrenoceptor activation by increased sympathetic outflow, but could also reflect inhibition of alpha-adrenoceptor activation by increased circulating levels of alpha-agonist catecholamines. Further studies are needed into the clinical importance of prevention of the low-flow syndrome, and of the potential of phenoxybenzamine to inhibit these other pathways.

In our experiments, we achieved a range of norepinephrine concentrations between 10$^{-3}$ and 10$^{-5}$ mol.l$^{-1}$ (a concentration of 2 ng.ml$^{-1}$ to 2 µg.ml$^{-1}$ in the organ bath). Ensinger found that plasma norepinephrine levels in healthy volunteers rose in a linear fashion with infusion rates, reaching 7.5 ± 1.1 ng.ml$^{-1}$ with infusion rates of 0.2 µg.kg$^{-1}$ min$^{-1}$ [31]. The higher end of this range of infusion rates is not uncommonly required in patients on cardiac intensive care units. Thus we used concentrations of norepinephrine up to approximately threefold higher than would be expected in vivo. We therefore believe that our model was clinically relevant in demonstrating inhibition of contraction to concentrations of norepinephrine that would occur in practice.

In our study, we used a phenoxybenzamine incubation solution at a concentration of 38 µg.ml$^{-1}$. The arteries were then washed free of unbound phenoxybenzamine. The recommended therapeutic dose for phenoxybenzamine is 1 mg.kg$^{-1}$ in 200 ml of physiological saline administered intravenously over 2 h (British National Formulary, September 1999), i.e. a 350 µg.ml$^{-1}$ solution infused systematically for a 70 kg patient. We would therefore expect no significant systematic effects in patients from our protocol for in vitro incubation of the radial artery with phenoxybenzamine.

A potential criticism of our study could be that the phenoxybenzamine in the organ bath destroyed the contractile apparatus of the vessel. However, as noted above, the concentration of phenoxybenzamine we used was one-tenth of that to which vessels are exposed at the site of infusion when the drug is used clinically. Furthermore, we showed that at a time 18 h after incubation with phenoxybenzamine when the response to norepinephrine remained completely abolished, two other contractile agonists (potassium and U46619) still brought about a contraction of a similar magnitude to that provoked prior to exposure of the arteries to phenoxybenzamine.

Patients undergoing coronary artery bypass graft surgery with a radial artery conduit commonly develop an early hypoperfusion syndrome which may be complicated by an early low cardiac output state [1–4]. Administration of high doses of exogenous inotropes may further enhance the propensity to conduit spasm. Our in vitro study suggests that a clinically feasible brief 20-min pre-incubation of the radial artery with a low concentration of phenoxybenzamine should be considered in future strategies aimed at preventing this syndrome. It is likely that alpha-adrenoceptor mediated contraction is one of several constrictor mechanisms. Effective prevention of spasm may need more than one relaxant agent. For example pre-treatment with phenoxybenzamine did not prevent contraction to either potassium chloride or a thromboxane agonist (U46619) applied 18 h later; however, neither did papaverine.
Nonetheless, in view of the well-described increase in circulating levels of catecholamines around the time of cardiac bypass, the importance of alpha-adrenoceptor antagonists should neither be ignored nor underestimated. An effective strategy for prevention of radial artery spasm should therefore include a vasodilator protocol sufficient for effective and prolonged inhibition of alpha-adrenoceptor mediated contraction. The observation that phenoxybenzamine also blocks a range of other constrictor pathways supports its use in this setting. Our findings strongly supports the inclusion of phenoxybenzamine along with, for example, other specific inhibitors of important vasoconstric tor mediators, such as thromboxane A2 antagonists in future strategies aimed at preventing this important syndrome.

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References