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Effect of mode of application of papaverine on the contractile response of the internal mammary artery

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A study was performed to investigate the duration of action of papaverine applied either intraluminally or in a combined intraluminal and extraluminal fashion *in vitro*, and how the reactivity of the internal mammary artery (IMA) to a range of vasoconstrictors is affected. Segments of IMA exposed to only intraluminal papaverine (10^{-4} mol/l) for 15 min recovered their contractile response to 90 mmol/l potassium chloride to pretreatment levels within 2 h. In contrast, combined intraluminal and extraluminal administration of papaverine resulted in a significant depression of the contractile response to 90 mmol/l potassium chloride ($P<0.001$), which persisted for at least 5 h. Responses to 5-hydroxytryptamine, noradrenaline, the thromboxane mimetic U46619, histamine and dopamine were not significantly different between control tissues and those that had received intraluminal papaverine. The duration of action of papaverine is affected by its route of delivery and there are no significant short-term effects on the contractile mechanisms in the arterial wall after intraluminal administration.

Perioperative changes in the vascular reactivity of coronary artery bypass grafts to vasoconstrictor and vasodilator stimuli could alter the balance of the mechanisms that govern vessel tone. This could result in generalized constriction or localized spasm of the graft (causing technical difficulties during the anastomosis) and/or a reduction in blood flow and therefore oxygen delivery to the myocardium, which is vulnerable to ischaemic damage during the perioperative period.

The effect of vasoconstrictor stimuli may be controlled by vasodilator therapy[1], the efficacy of which depends on the choice of drug and the method of administration. Papaverine, which inhibits phosphodiesterase and leads to increased intracellular guanosine 3',5'-cyclic monophosphate and adenosine 3',5-cyclic monophosphate concentrations[2–4], has been used as a pharmacological tool in this respect to improve the immediate performance of internal mammary artery (IMA) coronary bypass grafts[5–7]. Papaverine may be applied intraluminally via retrograde injection or by an extraluminal spray or swab. However, concern has been expressed over possible detrimental effects of papaverine when administered intraluminally[8]. Endothelial damage and an altered reactivity to vasoconstrictors might affect the short- and long-term performance of the graft.

To compare the effectiveness of papaverine in maintaining vasodilatation and to study the modulating effect of other vasoconstrictors, the response of isolated segments of IMA after selective intraluminal or combined intraluminal and extraluminal administration of papaverine *in vitro* was assessed.

Patients and methods

Specimens of IMA were obtained from 59 patients aged 31–74 years undergoing coronary artery bypass surgery Samples were placed immediately into a modified Tyrodes solution of the following composition: NaCl 136·9 mmol/l, $NaHCO_3$ 11·9 mmol/l, KCl 2·7 mmol/l, NaH_2PO_4 0·4 mmol/l, $MgCl_2$ 2·5 mmol/l, $CaCl_2$ 2·5 mmol/l, glucose 11·1 mmol/l and disodium ethylene diaminetetra-acetic acid 0·04 mmol/l. Arteries were transported to the laboratory and dissected free of connective tissue. They were then cut into 3–5-mm segments and placed into organ baths containing 5 ml modified Tyrodes solution. The baths were maintained at 37°C and continuously gassed with 95 per cent oxygen and 5 per cent carbon dioxide. Each ring segment was placed on two L shaped metal hooks, one of which was connected to a screw while the other was attached to an FTO-3C force–displacement transducer (Grass, Quincy, Massachusetts, USA). The transducers were connected to a Grass 79D polygraph to monitor and record changes in vessel wall tension.

A tension of 40 mN was applied to each vessel segment, which was allowed to relax over a period of 40–60 min. The Tyrodes solution was changed every 15–20 min during this period. Once a stable baseline had been attained, tissue viability and smooth muscle function were assessed on two occasions by addition to the baths of 90 mmol/l potassium chloride, the vessel segments being washed and allowed to return to baseline between each addition of the salt. A concentration of papaverine was chosen (10^{-4} mol/l) that was known[2] to be supramaximal and equates to the dose that is administered during surgery. The papaverine was diluted in normal saline.

To examine the time course of the effect of 10^{-4} mol/l papaverine on the IMA, segments were given either intraluminal or combined intraluminal and extraluminal drug. In one group of tissues the Tyrodes solution was drained from the baths and the papaverine (10^{-4} mol/l) administered directly into the lumen via a 25-G needle, the injection being performed such that the solution produced no distending pressure in the vessel segment but remained in contact with the vessel as a result of hydrostatic forces. In the second group, papaverine was added directly to the Tyrodes solution in the bath, giving a combined intraluminal and extraluminal administration. The difference in the effect on this group compared with that on the intraluminal group is assumed to be due to papaverine acting mainly on the extraluminal side of the vessel wall during the time of exposure. In both groups papaverine remained in contact with the vessel for 15 min. Control segments received normal saline, either added to the bath or injected into the lumen. After incubation the papaverine was removed by washing the tissues with fresh Tyrodes solution. A response to 90 mmol/l potassium chloride was then obtained. Once the maximum tension had developed the potassium chloride was removed from the bath by changing the Tyrodes solution twice; the vessel segments returned to the baseline state. Vessels were then left for a period of 1 h during which time the bath solution was changed three or four times. The response to 90 mmol/l potassium chloride was then retested. This procedure was continued for up to 5 h.

In a second series of experiments the reactivity of IMA segments was assessed by adding a range of vasoconstrictors. Intraluminal

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papaverine was administered to the vessels as described above. Once the response to 90 mmol/l potassium chloride had returned to its pretreatment value and a stable baseline had been attained, cumulative additions (in 0·5 $\log_{10}$ units) of noradrenaline, 5-hydroxytryptamine (5-HT), histamine, dopamine and the thromboxane mimetic U46619 were made. Control tissues received intraluminal saline and were treated in an identical manner to papaverine-treated vessels. Each vessel segment received only one agonist. Responses were measured as changes in tension and calculated as a percentage of the prepapaverine response to 90 mmol/l potassium chloride. Computed estimates of the effective concentration of agonist causing half-maximal contraction were found and expressed as the EC_{50} for each drug.

For the time-course experiments the potassium chloride response before papaverine was standardized to 100 per cent to allow direct comparison between responses before and after addition of the drug. Values are expressed as mean(s.e.m.); n denotes the number of patients from which samples were obtained and every individual treatment was performed on at least two segments from each patient. Statistical comparisons were made using Student's unpaired t test or one-way analysis of variance (ANOVA). $P<0·05$ was considered significant.

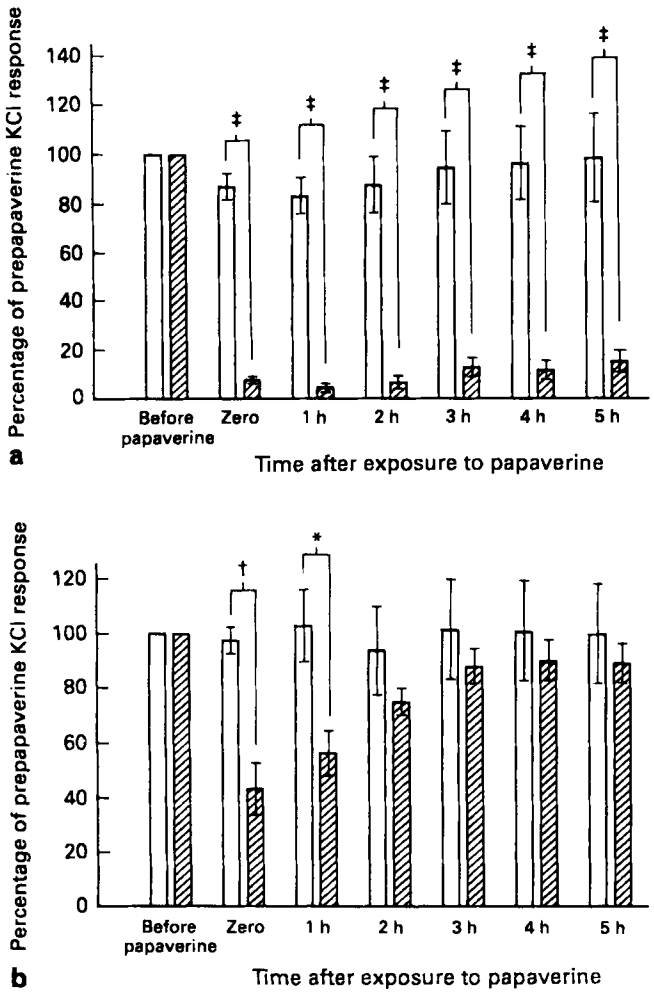

Fig. 1 a Contractile effect of repeated administration at 1-h intervals of 90 mmol/l potassium chloride after exposure to vehicle (□; $n=5$) or 10^{-4} mol/l papaverine injected into the bathing solution surrounding the vessels for 15 min (■; $n=5$). b Contractile effect of repeated administration at 1-h intervals of 90 mmol/l potassium chloride after exposure to vehicle (□; $n=4$) or 10^{-4} mol/l papaverine injected only into the lumen of the vessel segments for 15 min (■; $n=4$). Values are mean(s.e.m.). *$P<0·05$, †$P<0·01$, ‡$P<0·005$ (Student's t test)

Results

Time-course

Both intraluminal and combined intraluminal–extraluminal administration of 10^{-4} mol/l papaverine induced a relaxation of resting vessel segments. After the 15-min incubation with the drug there was an immediate attenuation in the response to 90 mmol/l potassium chloride, for both intraluminal and combined intraluminal–extraluminal papaverine. This significant attenuation in the contractile response of smooth muscle persisted for up to 5 h after administration of combined intraluminal and extraluminal papaverine ($n=5$) (*Fig. 1a*). In contrast, the effect caused by intraluminal papaverine ($n=4$) returned to control values after 2 h of intermittent washout (*Fig. 1b*).

Vascular reactivity

After incubation with intraluminal papaverine for 15 min and a 2-h washout period the reactivity of papaverine-treated ($n=4$) and control ($n=4$) segments of IMA was assessed with 90 mmol/l potassium chloride (*Fig. 2*). The return of the potassium chloride response to pretreatment levels indicated that any depressant action of the vasodilator on the smooth muscle contractile function had been removed. Both vehicle- and papaverine-treated segments of IMA responded with dose-dependent increases in tension after administration of noradrenaline ($n=6$), 5-HT ($n=6$), dopamine ($n=4$), histamine ($n=6$) and U46619 ($n=6$) (*Figs 3a–e*). There was no significant difference in the maximum response of all agonists tested

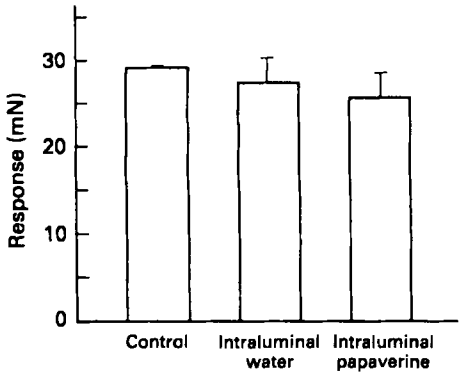

Fig. 2 Comparison of the mean(s.e.m.) response to 90 mmol/l potassium chloride after 2 h recovery in segments that had received either no treatment (control) ($n=4$), intraluminal distilled water ($n=4$) or 10^{-4} mol/l intraluminal papaverine ($n=4$)

Table 1 Maximum responses of 5-hydroxytryptamine, noradrenaline, dopamine, histamine and U46619 in the presence and absence of papaverine (10^{-4} mol/l) on the internal mammary artery

	Maximum response (mN)	
	Intraluminal saline	Intraluminal papaverine
5-hydroxytryptamine ($n=6$)	52·0	87·7
Noradrenaline ($n=6$)	70·1	80·3
Dopamine ($n=4$)	31·2	24·3
Histamine ($n=6$)	76·5	86·3
U46619 ($n=6$)	193·4	161·6

Values are means

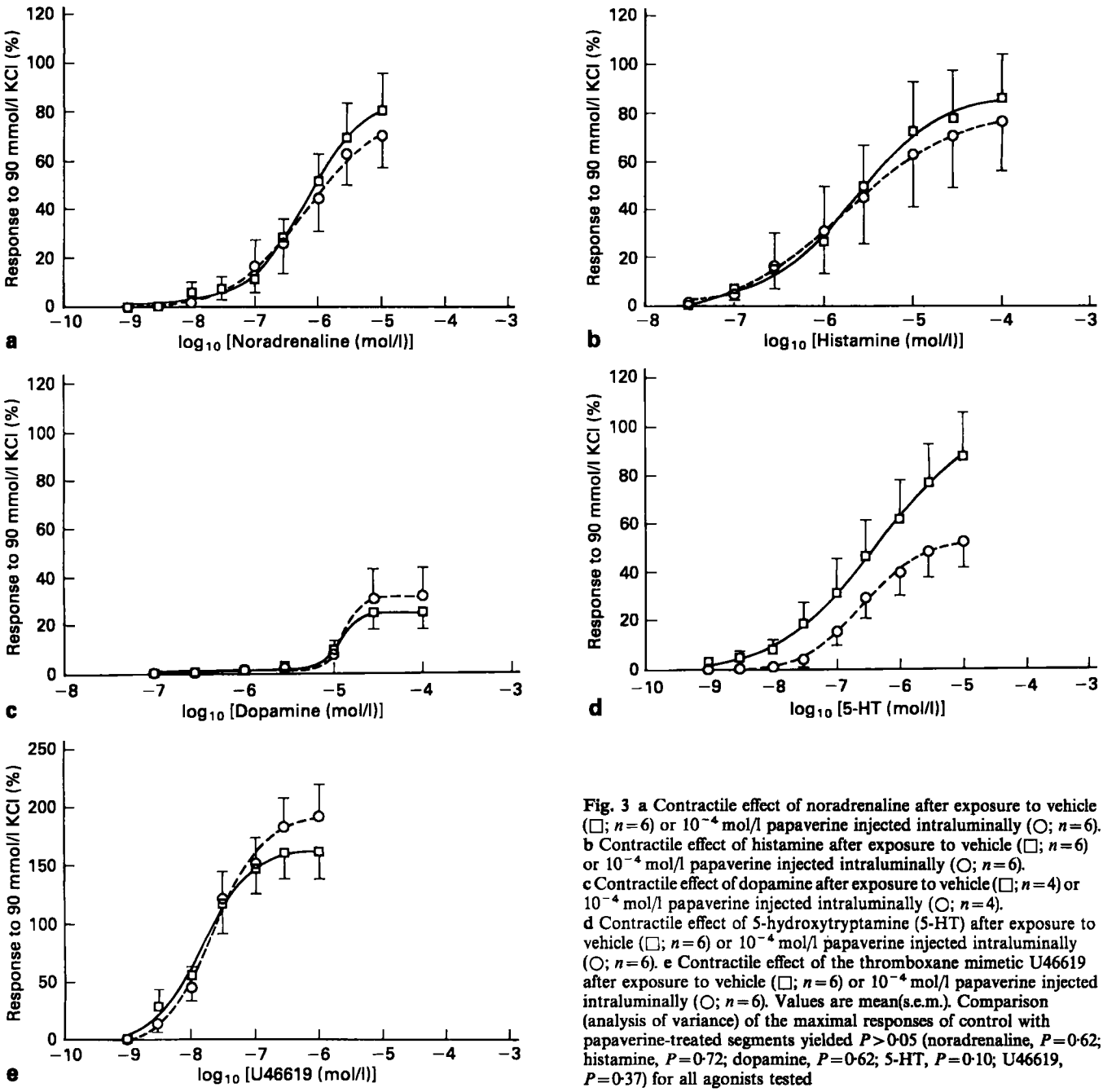

Fig. 3 a Contractile effect of noradrenaline after exposure to vehicle (□; $n=6$) or 10^{-4} mol/l papaverine injected intraluminally (○; $n=6$). b Contractile effect of histamine after exposure to vehicle (□; $n=6$) or 10^{-4} mol/l papaverine injected intraluminally (○; $n=6$). c Contractile effect of dopamine after exposure to vehicle (□; $n=4$) or 10^{-4} mol/l papaverine injected intraluminally (○; $n=4$). d Contractile effect of 5-hydroxytryptamine (5-HT) after exposure to vehicle (□; $n=6$) or 10^{-4} mol/l papaverine injected intraluminally (○; $n=6$). e Contractile effect of the thromboxane mimetic U46619 after exposure to vehicle (□; $n=6$) or 10^{-4} mol/l papaverine injected intraluminally (○; $n=6$). Values are mean(s.e.m.). Comparison (analysis of variance) of the maximal responses of control with papaverine-treated segments yielded $P>0.05$ (noradrenaline, $P=0.62$; histamine, $P=0.72$; dopamine, $P=0.62$; 5-HT, $P=0.10$; U46619, $P=0.37$) for all agonists tested

Table 2 Dose ratio values of 5-hydroxytryptamine, noradrenaline, dopamine, histamine and U46619 indicating no change in EC_{50} in the presence and absence of papaverine on the internal mammary artery

	EC_{50} intraluminal saline / EC_{50} intraluminal papaverine
5-hydroxytryptamine ($n=6$)	0·955
Noradrenaline ($n=6$)	0·871
Dopamine ($n=4$)	1·175
Histamine ($n=6$)	0·794
U46619 ($n=6$)	1·585

Values are ratios of mean EC_{50} values

between the vehicle- and papaverine-treated segments (*Table 1*). In addition, there was no effect on the EC_{50} of any drug after intraluminal exposure to papaverine (*Table 2*).

Similar experiments were not performed after combined intraluminal–extraluminal treatment as the response to 90 mmol/l potassium chloride did not return to pretreatment levels for up to 5 h.

Discussion

Papaverine has been used to treat perioperative spasm of the IMA[1,5]. The present study has demonstrated that the method of administration may profoundly affect the duration for which

this benefit may last. Combined intraluminal and extraluminal administration of papaverine affected smooth muscle from both the endothelial and adventitial surfaces and induced paralysis of the contractile mechanisms for at least 5 h. In contrast, *selective* administration of papaverine into the lumen of the vessel yielded an attenuation of the contractile response to potassium chloride for no more than 2 h.

Maintenance of the endogenous mechanisms that control vessel tone is thought to be important for a graft to function successfully[9,10]. Bypass grafts in which there is damage to the endothelium and/or smooth muscle may be associated with poor patency rates[11]. The satisfactory success rate of IMA grafts indicates that this artery retains the mechanisms that protect it against short- and long-term occlusion[12]. The success using the IMA is probably enhanced by the use of perioperative vasodilator drugs such as papaverine.

There are other drugs with similar actions, but there is debate about which agent is most effective. It has been shown that topical application of sodium nitroprusside can increase flow in the IMA by up to 250 per cent, compared with a 44 to 62 per cent increase with papaverine[1]. However, a recent comparative study[13] demonstrated that papaverine was the most efficacious inhibitor of potassium chloride- and noradrenaline-induced contraction of IMA segments. Jett *et al.*[13] also showed that vasodilators such as nitroglycerine, isoprenaline and adenosine were able to produce little or no inhibition of contraction, whereas nifedipine, verapamil and sodium nitroprusside were capable of producing pronounced inhibition of potassium chloride- and noradrenaline-induced contraction of segments of IMA. Further studies are required to assess comparatively the duration of action of the nitrovasodilators, calcium channel antagonists and other phosphodiesterase inhibitors in the protection of the vessel wall from vasoconstrictor stimuli.

The *in vitro* method of administration of papaverine used in the present study is not directly comparable to its use during coronary artery bypass surgery. When papaverine or other vasodilators are administered during surgery, they will gradually be cleared from the vessel wall by continuing flow through the artery. If flow is restricted, by occlusion of the distal end of the vessel with a clamp, the drug would indeed be allowed continued access to the vessel wall, but hard-jaw clamps cause loss of endothelial lining at the site of application[14]. In addition, increased pressure due to hydrostatic dilatation has detrimental histological effects on the IMA[15]. Care was taken in the design of the present study not to confound the results by allowing distending pressures to develop; indeed technically it was impossible for this to occur. The contribution of different distending pressures to the efficacy of papaverine requires investigation.

By subtracting the effect produced by intraluminal papaverine from that of the combined administration, it can be gauged what effect papaverine applied to the extraluminal surface only would have had (although some leakage from the end of the vessel segments would also have occurred in the intraluminal experiment). Perioperative administration of papaverine by soaking or spraying the outside of the vessel will probably allow a maximum effect of the drug well into the immediate postoperative period. This may be of significant benefit in the early performance of the graft. For the maximum advantage to be gained from papaverine the drug should be allowed to remain in contact with the vessel wall. This may be achieved by repeated administration of extraluminal papaverine during the operative period. In contrast, the effect of intraluminal papaverine can be expected to last for only a relatively short time. Papaverine should also act to modulate the response of the vessel wall to vasoconstrictor stimuli encountered during or immediately after surgery; substances such as thromboxane, 5-HT, histamine and catecholamines have all been shown to elicit varying degrees of vasoconstriction in human blood vessels[16–19].

In addition to its shorter duration of action, intraluminal papaverine might have detrimental effects on the endothelial and smooth muscle mechanisms that contribute to the control of vessel tone. This has recently been examined[8] in an experimental set-up similar to that used in the present study. Hillier and co-workers[8] showed that there was no difference in endothelial function, as judged by the relaxant response to acetylcholine and bradykinin, between segments of IMA that had received 5 min exposure to intraluminal papaverine compared with controls. The same study demonstrated a decreased activity of noradrenaline on segments that had received intraluminal papaverine. No significant change in the contractile response of the IMA to noradrenaline, 5-HT, histamine, dopamine or U46619 after treatment with papaverine was shown in the present study. This range of vasoconstrictors indicates that there is no change in vessel wall reactivity following exposure of the lumen to a high concentration of papaverine. The discrepancy between this result and the findings of Hillier *et al.*[8] with respect to noradrenaline may be due to insufficient recovery of the contractile mechanisms of the vascular smooth muscle in the papaverine-treated segments used in their study.

The present work has demonstrated that combined application of intraluminal and extraluminal papaverine produces a much longer-lasting relaxation of the vascular smooth muscle than that with intraluminal administration alone. In addition, there are no apparent immediate and lasting adverse effects on the vascular reactivity of the IMA following intraluminal administration of papaverine. To maximize the benefit of the drug, combined intraluminal–extraluminal administration of papaverine appears to be required.

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