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Autoradiographic localization and function of β-adrenoceptors on the human internal mammary artery and saphenous vein

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1 Receptor autoradiography with (−)-[^{125}I]-cyanopindolol (CYP) was used to study the distribution of β_1- and β_2-adrenoceptor subtypes in the human internal mammary artery and saphenous vein.

2 Images from X-ray film and nuclear emulsion coated coverslips, exposed to [^{125}I]-CYP labelled sections, showed a high density of β_2-adrenoceptors localized to the endothelium of the internal mammary artery and fewer β_2-adrenoceptors on the smooth muscle.

3 The function of β-adrenoceptors in ring preparations of the internal mammary artery was investigated in organ bath studies. (−)-Isoprenaline produced concentration-dependent relaxation of phenylephrine contracted rings. The potency and maximal effects of (−)-isoprenaline were not influenced by the presence of the endothelium.

4 Images of [^{125}I]-CYP binding to the saphenous vein, from X-ray film and nuclear emulsion coated coverslips, showed localization of β_2-adrenoceptors to the outer smooth muscle and not to the endothelium.

5 Relaxation of mammary artery and saphenous vein to (−)-isoprenaline is mediated via β_2-adrenoceptors located on the smooth muscle. Endothelial β_2-adrenoceptors, although present on the internal mammary artery, mediate other functions.

Introduction

The internal mammary artery and saphenous vein are currently used in coronary artery bypass grafting. Post-operative angiographic and epidemiological studies indicate that the internal mammary artery offers a better long-term patency and survival than the saphenous vein (Grondin *et al.*, 1984; Kamath *et al.*, 1985, Loop *et al.*, 1986). However, perioperative infarction, due to restriction of flow caused by spasm of the internal mammary artery, resistant to pretreatment with papaverine has been reported (Meldrum-Hanna & Ross, 1986; Buxton *et al.*, unpublished observations) and the incidence of cardiac related deaths in patients with internal mammary artery grafts is significantly greater than in patients with saphenous vein grafts (Buxton *et al.*, unpublished observations). Little is known about receptor distribution on these blood vessels and knowledge of receptor location may provide an understanding of the mechanism of spasm and suggest a rational approach for its reversal. Relaxation of many blood vessels is mediated by β-adrenoceptors and in this study autoradiography at the light microscopic level was used to determine the distribution of β_1- and β_2-adrenoceptors in sections of internal mammary artery and saphenous vein. The distribution of β_1- and β_2-adrenoceptors was compared to that previously described in human coronary arteries (Buxton *et al.*, 1987). Functional responses of internal mammary artery rings to stimulation of β-adrenoceptors were also tested in organ bath studies.

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Methods

Preparation and sampling of tissues

Tissues were obtained from patients undergoing coronary bypass surgery who had given informed consent to a protocol approved by the Epworth Hospital Ethics Committee. Patients routinely received papaveretum 15–20 mg and hyoscine hydrobromide 0.3–0.4 mg 1 h before preparation for anaesthesia which was induced with diazepam, phenoperidine or fentanyl and 66% N_2O in oxygen. Patients were paralysed with pancuronium and anaesthesia maintained with further doses of phenoperidine or fentanyl. Blood pressure was controlled with glyceryltrinitrate. The diagnoses and drug therapy of the patients studied are listed in Table 1. Tissues for autoradiographic processing were transported to the laboratory in a sealed flask at 4°C in a solution comprising equal parts sucrose 0.32 M and Krebs buffer (composition mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.27, Na_2PO_4 10.0, pH 7.4; containing Na heparin 50 iu ml^{-1}), whilst tissues for organ bath studies were transported in the same buffer used for these experiments (composition mM: NaCl, 112.1, KCl 5.0, $CaCl_2$ 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, glucose 11.5, KH_2PO_4 1.0, pH 7.4). Both solutions were equilibrated with 95% O_2 and 5% CO_2.

In the laboratory (15–20 min following surgical removal) blood vessels were divided and the intimal surface of one segment gently rubbed with roughened polyethylene tubing whilst the other was left intact. Blood vessels were frozen in isopentane previously cooled in liquid N_2. Sections (10 μm) were cut on a Reichert–Jung Cryostat at −20°C and mounted onto gelatin/chrome alum coated microscope slides (Young & Kuhar, 1979).

Table 1 Details of patients diagnosed with ischaemic heart disease undergoing coronary bypass surgery from whom tissue samples were obtained for this study

	Sex	*Age*	*Tissue*	*Concurrent drug therapy*
1*	F	66	MA SV	Verapamil, prazosin, glyceryltrinitrate
2	M	50	MA SV	Nifedipine, glyceryltrinitrate, temazepam
3	M	50	MA	Metoprolol, lorazepam, dipyridamole
4	M	49	MA	Diltiazem, glyceryltrinitrate, temazepam
5	M	55	MA	Nifedipine, metoprolol, diazepam
6	M	65	MA	Nifedipine, glyceryltrinitrate

Tissues samples; MA, internal mammary artery, SV, saphenous vein.
* 1–2 autoradiographic studies, 3–6 organ bath studies.

X-ray film and nuclear emulsion autoradiography

Slide-mounted sections were pre-incubated with 0.1 mM guanosine triphosphate (GTP) in Krebs buffer, containing 0.1 mM ascorbic acid and 10 μM phenylmethylsulphonylfluoride, for 30 min and then with the buffer and (−)-[^{125}I]-(cyanopindolol (CYP) (50–60 pM) at 25°C for 150 min in the absence and presence of the β_2-adrenoceptor selective antagonist ICI 118,551 (70 nM) (Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980), the β_1-adrenoceptor selective antagonist CGP 20712A (100 nM) (Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987) or (−)-propranolol (1 μM) to define non-specific binding.

Labelled sections were rinsed in buffer followed by 2 × 15 min washes at 37°C in the same medium and finally a rinse in distilled water at 22–25°C and dried in a stream of cold dehumidified air. Emulsion-coated coverslips (Kodak NTB3) were exposed to slide-mounted sections for 30 days, developed in Kodak Dektol, briefly rinsed in water and fixed in Kodak Rapid Fix at the paper dilution. Sections were stained with pyronine Y, dried and mounted for light microscopy. Every 10th section was stained with haematoxylin and eosin for histological examination. Alternatively X-ray film (Kodak NMC100) was exposed to dried labelled sections in light tight boxes for 75 days. The film was developed with Kodak D19, briefly rinsed in water, and fixed with Kodak Rapid Fix.

Organ bath studies

Segments of isolated mammary artery were used for experimentation either on the day of surgery or kept overnight in oxygenated buffer (see above for composition) at 4°C. The arterial segments were cleared of fat and connective tissue, cut into rings and endothelium removed from some rings using a moistened taper of filter paper. Rings were placed over two parallel wires and suspended under 2 g tension in organ baths containing buffer maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Rings were washed at 15 min intervals and tension readjusted to 2 g at the end of a 1 h equilibration period. Indomethacin (5 μM) was added to the buffer 10–15 min before contraction of the rings with phenylephrine and remained while relaxant responses to the various agonists were established (see below).

Sensitivity of the rings to the contractile agent phenylephrine was determined by adding increasing concentrations in a cumulative fashion (0.5 μM–10 μM). The rings were then washed until tension returned to basal levels. After 1 h tissues were contracted with a single concentration of phenylephrine which elicited a 1–2 g increase in tension and the presence or absence of functional endothelium assessed from relaxant responses elicited by acetylcholine (1 μM) and/or adenosine triphosphate (ATP; 30 μM). The cycle was then repeated using the cumulative addition of (−)-isoprenaline to relax the tissues two or three times until constant concentration-effect curves had been established. Dilutions of (−)-isoprenaline were made immediately before each curve to minimize oxidation. Relaxant responses to the agonists were expressed as a percentage of the phenylephrine contraction. For each ring a mean curve and pD_2 value for (−)-isoprenaline were calculated and these were used to calculate an overall mean curve and pD_2 value from 5–9 rings.

Statistics

Statistical differences between groups were determined by use of Student's *t* test for grouped data (Tallarida & Murray, 1981).

Drugs

The drugs used were: (−)-isoprenaline bitartrate (Wyeth); phenylephrine HCl; acetylcholine chloride; adenosine triphosphate (Sigma); indomethacin (Merck Sharpe & Dohme); (−)-propranolol

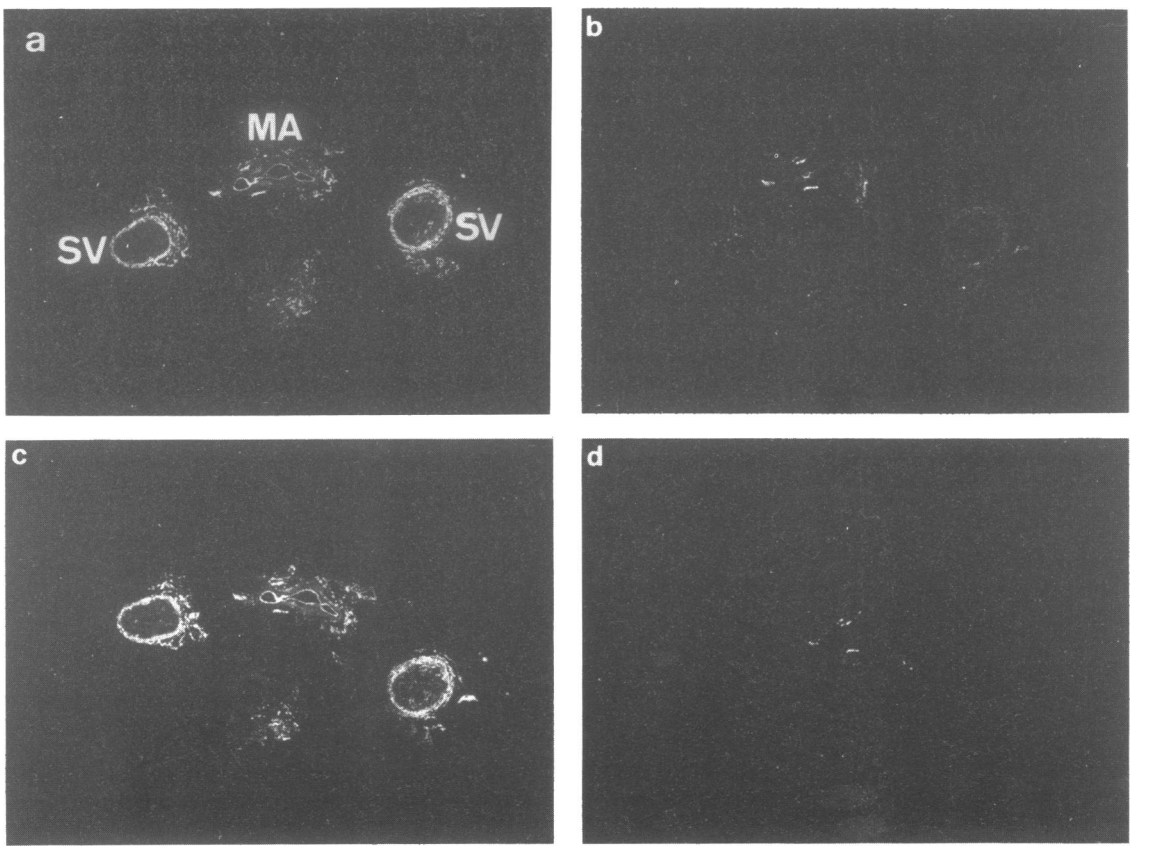

Figure 1 Photographs of X-ray film images showing the distribution of [^{125}I]-cyanopindolol ([^{125}I]-CYP) binding sites in serial sections of internal mammary artery (MA) and saphenous vein (SV). Sections were incubated with [^{125}I]-CYP (50 pM) in the absence (a) and presence of ICI 118,551 (70 nM) to show β_1-adrenoceptors (b) or CGP 20712A (100 nM) to show β_2-adrenoceptors (c) or (−)-propanolol (1 μM) to show non-specific binding (d). Note a high density of β-adrenoceptors localized to the intimal surface of the internal mammary artery and an even distribution of β-adrenoceptors on the medial smooth muscle of the saphenous vein (a) which were of the β_2-subtype, since binding was absent in the presence of ICI 118,551 (b) and present in the presence of CGP 20712A (c). Localized binding of [^{125}I]-CYP to patches of adventitia were observed in the presence of (−)-propranolol (d), which was to dense aggregates of nuclei clustered in the lumen of small blood vessels.

(autoradiography experiments); (±)-propranolol (organ bath experiments); ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) (Imperial Chemical Industries); CGP 20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifuoromethyl)1H-imidazole-2-yl)-phenoxy)propyl)amino)-ethoxy)-benzamide monomethane sulphonate) (Ciba-Geigy); $Na^{125}I$ (Amersham International); (−)-cyanopindolol (Sandoz, Basle); (−)-[^{125}I]-CYP was prepared from (−)-CYP and $Na^{125}I$ as previously described (Lew & Summers, 1985); guanosine triphosphate (Boehringer Mannheim); NTB3 nuclear emulsion, Rapid Fix, D19, Dektol (Kodak); pyronine Y, haematoxylin (Sigma); eosin (Medos).

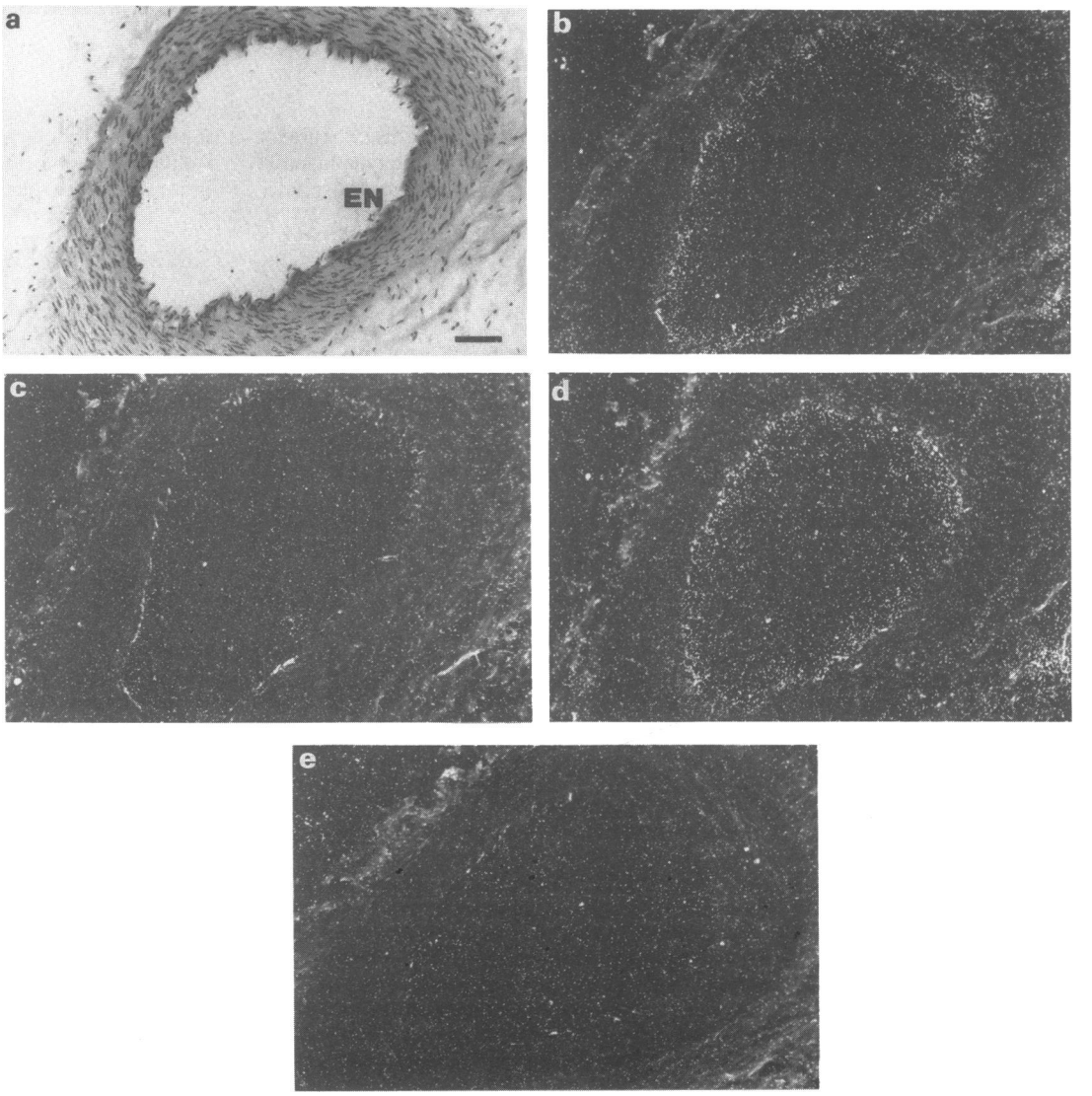

Figure 2 Autoradiographic localization of [^{125}I]-cyanopindolol ([^{125}I]-CYP) binding to human internal mammary artery. (a) Shows a photomicrograph of a haematoxylin and eosin stained section of internal mammary artery with intact endothelium (EN). (b)–(e) Darkfield micrographs of serially cut sections incubated with [^{125}I]-CYP in the absence (b) and presence of 70 nM ICI 118,551 (c), 100 nM CGP 20712A (d) or 1 μM (−)-propranolol (e) and apposed to nuclear emulsion coated coverslips. In (b) there was a high density of β-adrenoceptors localized to the endothelium which were of the β_2-subtype (d). There was a lower density of β-adrenoceptors on the smooth muscle (b) which were also of the β_2-subtype (d). Bar represents 100 μm.

Stock solutions (10 mM) of CGP 20712A, ICI 118,551 and (−)-isoprenaline were prepared in 0.01 M HCl. Indomethacin was prepared as a 10 mg ml^{-1} solution in 0.5% w/v Na_2CO_3. Acetylcholine chloride (0.1 M) was dissolved in 0.1 M NaH_2PO_4 and the remaining drugs in distilled water. Dilutions were made using the appropriate Krebs buffer. All other chemicals were of analytical grade.

Results

X-ray film and nuclear emulsion coated coverslips were exposed to sections labelled with [^{125}I]-CYP in the absence and presence of ICI 118,551 (70 nM) (β_2-adrenoceptor selective) or CGP 20712A (100 nM) (β_1-adrenoceptor selective) or (−)-propranolol (1 μM) to define non-specific binding. The images obtained revealed the distribution of β-adrenoceptor binding sites over the internal mammary artery and saphenous vein. The photographs were taken from X-ray film images and reveal a high density of sites on the smooth muscle of the saphenous vein, a low density over the media of the internal mammary artery, and highly localized binding to the intimal surface of the internal mammary artery (Figure 1a). Competition with ICI 118,551 (Figure 1b) and CGP 20712A (Figure 1c) demonstrated that these were predominantly of the β_2-adrenoceptor subtype (Figure 1c). Binding to the smooth muscle of both blood vessels and to the intimal surface of the internal mammary artery was abolished by (−)-propranolol (1 μM) (Figure 1d). Highly localized binding to patches of adventitia was also observed which was (−)-propanolol-insensitive. Examination of corresponding haematoxylin and eosin stained sections at greater magnification showed that these sites were unusually dense aggregates of nuclei clustered in the lumen of small blood vessels (data not shown).

Greater detail and higher resolution was obtained by exposing nuclear emulsion coated coverslips to [^{125}I]-CYP-labelled sections. Good anatomical correlation was obtained between serially cut sections, which had been stained with haematoxylin and eosin, and those labelled with [^{125}I]-CYP and stained with pyronine Y. There was a high density of β-adrenoceptors localized to the endothelium of the internal mammary artery (Figure 2a, b) which were of the β_2-subtype since binding was abolished by ICI 118,551 (70 nM) (Figure 2c) and still evident in the presence of CGP 20712A (Figure 2d). In sections which had been gently rubbed with polyethylene tubing to remove the endothelium there was no localization of binding to the intimal surface (Figure 3b).

The role of β_2-adrenoceptors on the endothelium of the internal mammary artery in relaxation was

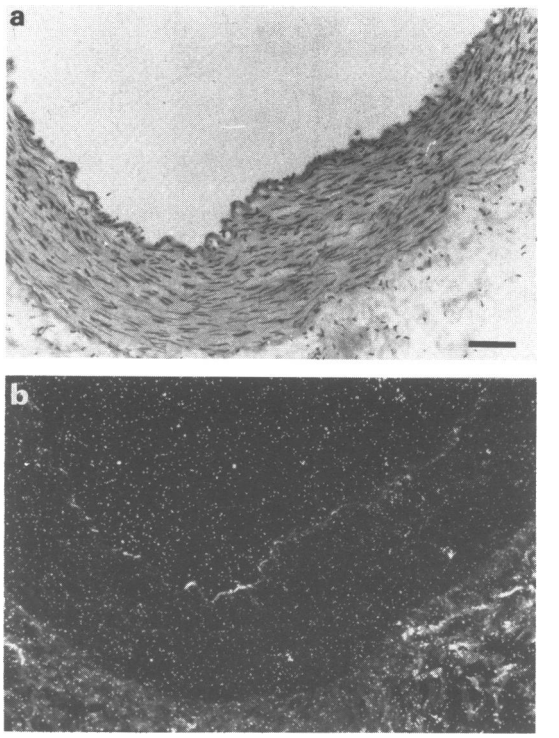

Figure 3 Effect of removal of the endothelium from the internal mammary artery on β-adrenoceptor binding. (a) Shows a photomicrograph of a haematoxylin and eosin stained section of internal mammary artery which was gently rubbed with polyethylene tubing to remove the endothelial cell surface. (b) Dark-field photomicrograph of [^{125}I]-cyanopindolol binding. Note the absence of localized β-adrenoceptor binding to the intimal surface in (b). Bar represents 100 μm.

examined in organ bath experiments. Rings of artery with (+E) and without endothelium (−E) were contracted with phenylephrine. The presence of functionally intact endothelium was tested using acetylcholine (1 μM) and or ATP (30 μM). Both agents produced similar relaxations of the mammary artery ($76 \pm 4\%$, $n = 9$) in rings with endothelium but failed to relax rings without endothelium. (−)-Isoprenaline produced propranolol (0.5 μM)-sensitive concentration-dependent relaxant responses in both preparations. Figure 4 shows traces from one experiment using a phenylephrine precontracted ring with endothelium in which responses to acetylcholine and (−)-isoprenaline in the absence and presence of propranolol were established. There was no difference in the potency, or the maximal response ($P > 0.05$, Student's t test), to (−)-isoprenaline in internal mammary artery preparations from patients

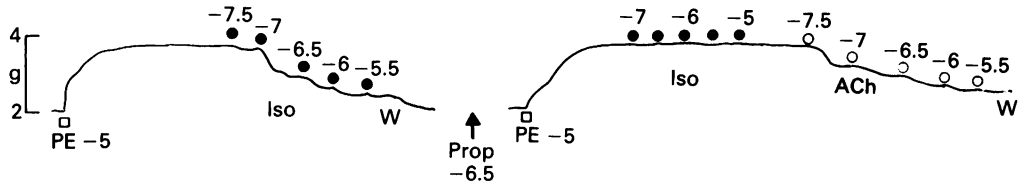

Figure 4 Trace from an experiment in which responses to (−)-isoprenaline (Iso) were established in phenylephrine (PE) precontracted rings with endothelium in the absence and presence of propranolol (Prop) (0.5 μM, 15 min). Also shown is a cumulative concentration-effect curve to acetylcholine (ACh) indicating the presence of a functional endothelium. Concentrations of drugs are expressed as the logarithm of concentrations.

who had or had not been treated with metoprolol to alleviate the symptoms of ischaemic heart disease. Therefore, the metoprolol-treated group were included in subsequent analyses. The potency of (−)-isoprenaline ($pD_2(+E) = 6.73 \pm 0.24$, $n = 9$; $pD_2(-E) = 6.55 \pm 0.29$, $n = 5$) and the extent of relaxation ($+E = 74.7 \pm 9.4\%$, $n = 9$; $-E = 80.52 \pm 10.5\%$, $n = 5$) were similar (Student's t test) in artery rings with and without endothelium. Figure 5 shows mean concentration-effect curves for relaxation to (−)-isoprenaline in phenylephrine precontracted internal mammary artery rings.

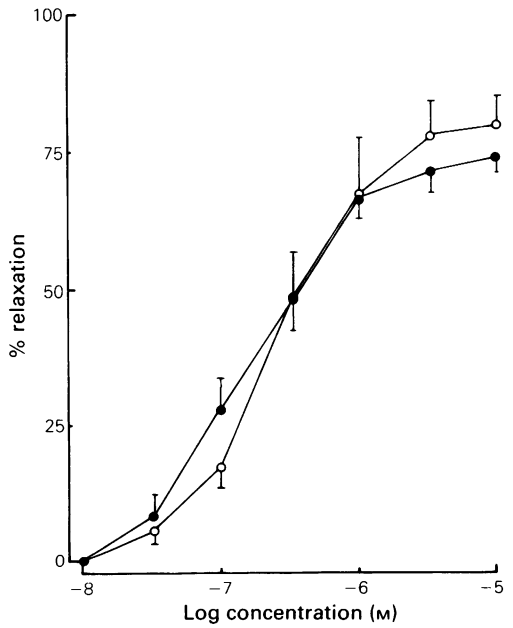

Figure 5 Mean cumulative concentration-response curves to (−)-isoprenaline in the internal mammary artery with (●) and without (○) intact endothelium. Rings were contracted with phenylephrine and relaxant responses expressed as a percentage of the phenylephrine-induced contraction. There was no difference in the potency and maximal response to (−)-isoprenaline in each tissue ($P > 0.05$, Student's t test). Points shown are means from 9 (with intact endothelium) and 5 (without endothelium) individual experiments; vertical lines indicate s.e. mean.

β_2-Adrenoceptors were also localized to the outer smooth muscle of the saphenous vein (Figure 6) and unlike the internal mammary artery they were localized only to this area. In one recent organ bath study using highly selective agonists and antagonists (Ikezeno *et al.*, 1987), it was shown that the β-adrenoceptor which mediates relaxation is of the β_2-subtype.

Discussion

The identification of receptors by receptor autoradiography has several advantages over functional techniques in terms of sensitivity and identification of receptor location. This information can be useful in determining function which can then be tested by conventional techniques. We have used [^{125}I]-CYP to label β_1- and β_2-adrenoceptors (Buxton *et al.*, 1987; Molenaar *et al.*, 1987). The highly selective β-adrenoceptor antagonist CGP 20712A, which has approximately 10,000 fold selectivity for the β_1-adrenoceptor (Dooley & Bittiger, 1984; Dooley *et al.*, 1986; Kaumann, 1986; Molenaar & Summers, 1987), and ICI 118,551, which has approximately 200 fold selectivity for the β_2-adrenoceptor (Bilski *et al.*, 1980; 1983; O'Donnell & Wanstall, 1980; Molenaar *et al.*, 1987), were used to identify the β-adrenoceptor subtypes.

β-Adrenoceptor localization in the human internal mammary artery and saphenous vein has been examined for the first time and can now be compared to that previously shown on native coronary arteries (Buxton *et al.*, 1987). The localization of β_2-adrenoceptors on the endothelium of the internal mammary artery is similar to that previously observed on native coronary arteries (Buxton *et al.*, 1987). However, the function of these β_2-adrenoceptors is unclear and organ bath studies were performed to determine whether they influence

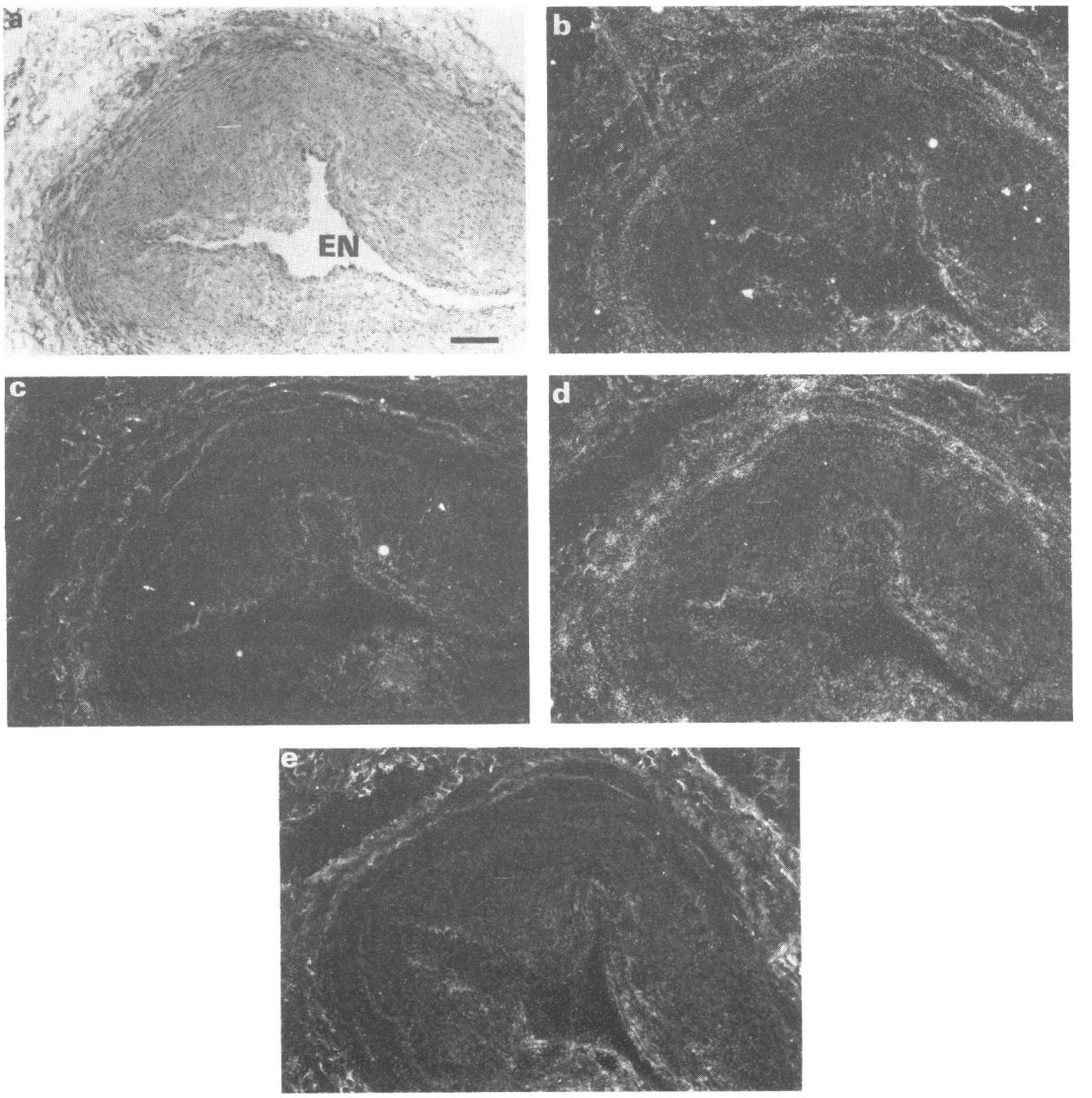

Figure 6 Autoradiographic localization of [^{125}I]-cyanopindolol ([^{125}I]-CYP) binding to human saphenous vein. (a) Shows a photomicrograph of a haematoxylin and eosin stained section of saphenous vein with intact endothelium (EN). (b)–(e) Darkfield micrographs of serially cut sections incubated with [^{125}I]-CYP in the absence (b) and presence of 70 nM ICI 118,551 (c), 100 nM CGP 20712A (d) or 1 μM (−)-propranolol (e) and apposed to nuclear emulsion coated coverslips. In (b) note β_2-adrenoceptors were only localized to the outer smooth muscle of the saphenous vein (d). Bar represents 200 μm.

the response to β-adrenoceptor agonists. (−)-Isoprenaline produced concentration-dependent relaxant responses in phenylephrine precontracted rings of mammary artery. The potency and maximal effect was not changed by careful removal of endothelium even though this was also shown to have the capacity to release EDRF to acetylcholine. Thus β_2-adrenoceptors on the endothelium of the mammary artery do not mediate release of EDRF. The low density of β_2-adrenoceptors on the smooth muscle is probably responsible for the relaxant responses to (−)-isoprenaline. Responses of the internal mammary artery to (−)-isoprenaline were not altered in patients pretreated with metoprolol, a

β-antagonist with 40–50 fold selectivity for the β_1-adrenoceptor (McPherson *et al.*, 1984).

Whilst coronary bypass utilizing the internal mammary artery is associated with a better long-term patency and survival than the saphenous vein, post-operative spasm which is resistant to pretreatment with papaverine can pose a serious problem. The maintenance of a functionally intact endothelium during surgical preparation for coronary bypass grafting may allow the development of a clinically useful dilator which acts through the release of relaxing factors such as NO (Palmer *et al.*, 1987). It would also be advantageous to use a drug which specifically dilates the internal mammary artery and therefore avoids peripheral vasodilatation and reduction in coronary perfusion pressure.

The localization of β-adrenoceptors on the saphenous vein differed from that in the internal mammary artery and coronary artery (Buxton *et al.*, 1987). Localized areas of β_2-adrenoceptors were found only on the outer smooth muscle. During surgical preparation of the saphenous vein for coronary bypass grafting the vein is commonly dilated with papaverine. In this study the tissue had not been dilated with papaverine and retained an intact endothelium. In these preparations β_2-adrenoceptors were not found on the endothelium. Studies using selective β_1- and β_2-adrenoceptor agonists and antagonists (Ikezono *et al.*, 1987) have shown that β_2-adrenoceptors mediate relaxant responses to β-adrenoceptor agonists and our studies would suggest that these are located on the outer smooth muscle layer.

In conclusion, the development of drugs to reverse spasm of the internal mammary artery post-operatively is required. Receptor autoradiography can provide information on receptor location and suggest strategies for the development of clinically useful drugs. In this study the technique has been used to determine the location of β_1- and β_2-adrenoceptors on the internal mammary artery and saphenous vein. The function of β-adrenoceptors on the internal mammary artery was also investigated in organ bath experiments, where it was shown that only the β-adrenoceptors on the smooth muscle mediate the relaxant responses to β-agonists.

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