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Substance P for Evaluation of Coronary Endothelial Function After Cardiac Transplantation

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The endothelium-dependent vasodilator substance P dilates normal and diseased coronary vessels in humans in vivo and produces a maximal response similar to that seen with intracoronary isosorbide dinitrate. Twelve cardiac transplant recipients underwent intracoronary infusion of substance P after routine annual investigations. All patients were well, with no evidence of rejection and with angiographically normal coronary arteries. Substance P was infused at 2 ml/min for 2 min into the coronary artery, starting at a dose of 1.4 pmol/min and increasing by doubling increments, and followed by isosorbide dinitrate (1 mg/min) infused over 2 min. Coronary artery diameter was measured in 23 vessel segments from 12 transplant recipients.

The following doses were infused: saline solution (1 ml/min), substance P (0.7 [three patients], 1.4, 2.8, 5.6, 11.2, 22.4 pmol/min) and isosorbide dinitrate (1 mg/min). The mean percent increase in diameter (± SEM) in response to increasing doses of substance P was as follows: 0, 6.5 ± 2.9%, 10.9 ± 2.9%, 12.1 ± 2.9%, 16.5 ± 2.6%, 19.2 ± 3.1% and 25.8 ± 2.2%, respectively. Half maximal dilation was produced with 1.4 to 2.8 pmol/min of substance P; the maximal response (mean percent diameter change) was 22 ± 2.5%. This was not significantly different from that achieved with isosorbide dinitrate.

It is concluded that coronary endothelial function as assessed by response to substance P is preserved in cardiac transplant recipients with angiographically normal coronary arteries. Substance P may be a suitable agent for testing endothelial function in these patients.

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The most important determinant of long-term prognosis after cardiac transplantation is the development of accelerated coronary artery disease (1). The normal physiologic behavior of the coronary arteries may be altered after cardiac transplantation, as a result of several possible mechanisms including denervation (2) and injury to the endothelium by the cardioplegic solution during the period of ischemic arrest (3). In addition, immunologically mediated injury to the endothelium (4) may occur and this process of endothelial damage has been postulated to be involved in the development of accelerated coronary artery disease.

Endothelial cells have been shown to be obligatory in the relaxation of smooth muscle by acetylcholine (5) and in vivo intracoronary studies have been carried out using this agent. It has been demonstrated (6) that in disease states such as atherosclerosis there is paradoxic vasoconstriction in response to acetylcholine, whereas normal coronary vessels respond with dilation, suggesting that there may be endothelial dysfunction in patients with atherosclerosis. In cardiac transplant recipients, similar studies (7) using intracoronary acetylcholine have suggested that there may be endothelial dysfunction in the coronary arteries with graft sclerosis. Other investigators (8) have shown a vasoconstrictor response to intracoronary acetylcholine early after cardiac transplantation and have questioned the link between the acetylcholine response and endothelial dysfunction in graft coronary sclerosis. In vitro investigations (9) of isolated coronary arteries have also shown that acetylcholine may not always relax these vessels and, when infused directly, may cause vasoconstriction in vivo in the majority of cases (10).

Substance P is an 11 amino acid vasodilator neuropeptide. It is found in the peripheral nervous system in sensory neurons (11), the vagus (12) and some sympathetic ganglia (13). It has also been localized to the perivascular nerves of many animals (14) and the perivascular regions of small arterioles in the human heart (15). It is a potent vasodilator in humans, causing vasodilation in the forearm when infused into the brachial artery (16) and dilating epicardial coronary arteries when infused into the coronary circulation (17). The vasodilative action of substance P has been shown to be endothelium dependent in most species (18), including humans (19,20), and its action in human coronary artery rings is abolished by the nitric oxide synthesis inhibitor

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Table 1. Clinical Characteristics of 12 Patients Undergoing Infusion of Substance P After Cardiac Transplantation

Pt. No.	Age (yr)	Donor Age (yr) Gender	Months After Transplant	Ischemic Time (min)	LV Function (EF %)	Treated Rejection Episodes	Blood Pressure (mm Hg)	Exercise Capacity (W)	Lipids (mmol/liter) TC	Lipids (mmol/liter) TG	HLA Matching mm	HLA Matching m	Maximal Response to Substance P (%)
1	49	19/M	60	100	77	3	140/90	No test	6.6	1.8	5	0	25.38
2	47	35/M	12	100	70	1	130/90	80	7.5	2.0	4	2	21.65
3	53	16/M	24	215	59	2	140/90	130	7.5	2.2	4	2	23.73
4	53	18/M	36	110	78	10	140/90	No test	5.1	1.2	—	—	8.98
5	63	18/M	24	164	78	3	130/80	No test	6.0	3.4	5	1	13.09
6	46	21/M	24	160	68	3	130/80	No test	7.7	1.5	—	—	23.08
7	55	41/M	48	180	76	3	150/90	90	9.1	1.6	5	0	24.79
8	53	18/M	12	157	68	1	130/80	110	5.4	2.7	4	0	36.96
9	51	17/M	12	165	65	4	130/90	50	5.2	1.1	6	0	48.91
10	55	26/M	60	192	74	3	130/85	No test	7.7	1.1	—	—	22.32
11	59	20/M	60	105	74	8	130/80	No test	7.0	2.5	5	1	30.09
12	50	18/M	48	215	67	2	130/90	130	6.3	2.2	—	—	16.03

EF = ejection fraction; F = female; HLA = human leukocyte antigen; LV = left ventricular; M = male; *m* = number of positive matches; *mm* = number of mismatches; Pt. = patient; TC = total cholesterol; TG = triglycerides.

L-N^{G}-monomethyl-arginine (21). These studies therefore indicate that substance P is a potent stimulant of endothelium-derived relaxing factor production by endothelium. The aim of this study was to investigate the use of substance P in the evaluation of coronary endothelial function in patients with angiographically normal coronary arteries after cardiac transplantation.

Methods

Study patients (Table 1). Twelve men were studied 1 to 5 years (mean 35 months) after cardiac transplantation. The mean age was 53 years (range 47 to 63). All patients had angiographically normal coronary arteries, which was the only selection criterion; they were not preselected. At the time of routine annual investigation, all were clinically well, with no evidence of cardiac rejection as assessed by endomyocardial biopsy. Table 1 lists the clinical characteristics of the 12 patients and includes patient and donor age, the time after transplantation at which the study was carried out, the number of treated rejection episodes and the ischemic times of the donor heart.

Infusion protocol. Substance P infusion was carried out after right heart catheterization, left venticulography, selective coronary angiography and right ventricular endomyocardial biopsy. Coronary arteriography was performed with the Judkins technique. A baseline angiogram performed with Omnipaque 350 (iohexol) was obtained in the projection best displaying the coronary anatomy, as determined previously from the diagnostic films. This position was not altered throughout the course of the study. A high resolution 12.7 cm image intensifier (Optimus M200 Phillips) was used. After a control angiogram was obtained, saline solution was infused at 1 ml/min for 2 min. This vehicle infusion was carried out before the infusion of substance P in all 12 patients. Increasing concentrations of substance P were then infused at a starting dose of 0.7 pmol/min in three patients and of 1.4 pmol/min in nine patients. Immediately after the infusion, another coronary angiogram was performed. A dose of substance P twice as large as the previous dose was then infused at the same infusion rate. Angiograms were repeated and this procedure was continued to a maximal dose of 22.4 pmol/min in 10 patients and of 11.2 pmol/min in 2 patients. On completion of the substance P infusions, isosorbide dinitrate was infused at 1 mg/min for 2 min, when a final angiogram was taken. In 11 of the 12 patients, substance P was infused into the left coronary artery and in 1 patient it was infused into the right coronary artery.

Ethics. Informed written consent was obtained from each patient. The study was approved by the Ethical Committee of Harefield Hospital and the Research and Ethical Committees of Hammersmith Hospital.

Quantitative angiographic analysis. Coronary artery luminal diameter was measured by an automated edge detection computer analysis system (Cardiovascular Angiography Analysis System, CAAS, Pie Data Medical). End-diastolic film frames were used for analysis and specific proximal segments of the left anterior descending and circumflex coronary arteries were chosen. A point after the first diagonal branch of the left anterior descending artery and after the first obtuse marginal branch of the left circumflex artery was used and the diameter across this point was measured. The size of the coronary catheter (7F in all cases) was used for calibration of the image in millimeters and correction was made for radiographic pincushion distortion. In all, 23 coronary vessel segments were analyzed: 11 left anterior descending artery segments, 11 left circumflex artery segments

and 1 right coronary artery segment (in the patient who had the right coronary artery infusion). Six of the 12 films were reanalyzed by a second observer and the measurement was highly reproducible ($r = 0.983$, p = NS between observations).

Data presentation. Coronary diameter is expressed in millimeters or as percent diameter change from the control value. Mean values ± 1 SEM are given for each dose of substance P. Responses were compared by using repeated measurements analysis of variance. A p value <0.05 was taken to indicate significance.

Results

The infusion studies were well tolerated by all patients, with no adverse effects during substance P or isorbide dinitrate infusion. No patient experienced symptoms during any stage of the infusions. No alteration in the surface electrocardiogram was seen.

Epicardial dilation (Table 2). The initial infusion of 0.9% *N* saline solution caused no significant change in coronary diameter compared with that determined with the baseline vehicle infusion. Substance P caused a dose-dependent increase in proximal luminal diameter (Fig. 1). In 15 vessel segments, the maximal response occurred at the two maximal doses of substance P, but in the remaining 8 segments the maximal response occurred at lower doses of 2.8 and 5.6 pmol/min; half maximal dilation was produced with 1.4 to 2.8 pmol/min. The mean maximal percent dilation achieved with substance P was not significantly different from that achieved with isosorbide dinitrate (22.02 ± 2.46% vs. 25.76 ± 2.17%) (Fig. 1). The mean preinfusion diameter was 2.98 ± 0.12 mm. For the total group, there was a significant difference between baseline vehicle infusion, lower doses of substance P and the 11.2 and 22.4 pmol/min doses of substance P. Analysis of individual patient maximal response to substance P and isosorbide dinitrate, however, showed that there were six vessel segments in six patients whose maximal substance P response was attenuated in comparison with the response to isorbide dinitrate (Fig. 2).

Peripheral hemodynamic effects. There was no significant change in heart rate with increasing doses of substance P and isosorbide dinitrate. Femoral artery systolic pressure showed a tendency to decrease at higher doses of substance P that reached significance at the 11.2 and 22.4 pmol/min level ($p < 0.05$) compared with the lower doses (Fig. 3). The mean maximal decrease in systolic pressure was 11.25 mm Hg. There was no further significant decrease in aortic systolic pressure after infusion of isorbide dinitrate. There was a similar trend for aortic diastolic pressure, with a significant difference between the 2.8 pmol/min dose and the two maximal doses (11.2 and 22.4 pmol/min) of substance P ($p < 0.05$). The mean maximal decrease in aortic diastolic pressure, 5.63 mm Hg, occurred at 22.4 pmol/min. Aortic diastolic pressure was unaltered by infusion of isosorbide dinitrate.

Follow-up angiography. Five patients (Patients 1 to 5) have now been followed up for routine annual investigation 1 year after the substance P infusion study. In these patients, the coronary arteries remain normal, with no detectable change in coronary artery dimensions.

Discussion

This study has shown that cardiac transplant recipients have a preserved vasodilator response to intracoronary infusion of substance P. The degree of maximal vasodilation observed in large epicardial vessels is similar in degree to the vasodilation observed in normal patients after infusion of intracoronary substance P and nitrates (17). Furthermore, in our patients, there was no relation between the length of time after cardiac transplantation and the degree of response to substance P, suggesting that the coronary endothelium remains a physiologically active organ many years after transplantation.

Comparison with other studies. A recent in vitro study (21) of human epicardial coronary rings indicated that relaxation induced by substance P is mediated by endothelium-derived relaxing factor because relaxation of coronary rings was inhibited by L-N^G-monomethyl-arginine (L-NMMA), which is a specific inhibitor of nitric oxide formation from its precursor L-arginine. Nitric oxide is now thought to be endothelium-derived relaxing factor (22). These observations suggest that the vasodilation observed in response to substance P in our patients is due to the release of endothelium-derived relaxing factor. In contrast, it was previously suggested (7) that endothelial dysfunction, to a degree that suggests a lack of endothelium-derived relaxing factor release, is an early finding after cardiac transplantation as shown in studies with intracoronary acetylcholine. Acetylcholine has been demonstrated to be a dilator of coronary arteries in vivo and in vitro in the presence of an intact endothelium. However, different studies (7,8) of the response of coronary arteries in cardiac transplant recipients to intracoronary acetylcholine have shown varying results. Fish et al. (7) demonstrated that in the majority of patients with angiographically smooth coronary arteries, the arteries failed to dilate or displayed paradoxic vasoconstriction in response to acetylcholine. It was implied that endothelial dysfunction was present and that there is some causal relation to the development of coronary atherosclerosis in these patients. More recently, it was shown (23) that acetylcholine may have a much greater effect on coronary blood flow than on epicardial coronary diameter and that it tends to cause coronary vasoconstriction in the majority of patients, even those with normal coronary arteries. Although studies in vitro (9,19) and in vivo (6) have demonstrated a dilative response of epicardial coronary arteries to acetylcholine,

Table 2. Absolute Diameter and % Diameter Change in 12 Patients Undergoing Infusion of Substance P After Cardiac Transplantation

Pt. No.	Substance P Dose (pmol/min)	LAD Diam (mm)	LAD % Diam	LCx Diam (mm)	LCx % Diam	RCA Diam (mm)	RCA % Diam*
1	Saline	2.85	0	2.60	0		
	0.7						
	1.4	2.79	−2.11	2.58	−3.08		
	2.8	2.94	3.16	3.08	18.46		
	5.6	3.37	18.25	2.89	11.15		
	11.2	3.21	12.63	3.25	25.00		
	22.4	3.38	18.66	3.26	25.38		
	ISDN	3.42	20.00	3.18	22.31		
2	Saline	4.03	0	3.28	0		
	0.7	3.32	−17.62	2.84	−13.41		
	1.4	3.76	−6.70	2.92	−10.98		
	2.8	4.15	2.98	3.35	2.13		
	5.6	3.85	−4.47	3.59	21.65		
	11.2	4.43	10.20	3.66	11.59		
	22.4	3.72	−7.69	3.62	10.37		
	ISDN	4.64	15.14	4.14	26.22		
3	Saline	2.95	0	2.87	0		
	0.7						
	1.4						
	2.8	3.28	11.90	3.01	4.88		
	5.6	3.65	23.73	2.80	−2.44		
	11.2	3.57	21.02	3.31	15.33		
	22.4						
	ISDN	3.83	29.83	3.67	27.87		
4	Saline	4.12	0	2.40	0		
	0.7	3.96	−3.88	2.49	3.75		
	1.4	4.42	7.28	2.38	−0.83		
	2.8	4.46	8.35	2.48	3.33		
	5.6	4.49	8.98	2.41	0.42		
	11.2	4.23	2.67	2.42	0.83		
	22.4						
	ISDN	4.32	4.85	2.96	23.33		
5	Saline	3.80	0	2.52	0		
	0.7						
	1.4	4.00	5.26	2.32	−7.94		
	2.8	4.35	14.47	2.02	−19.84		
	5.6	4.37	15.00	2.48	−1.59		
	11.2	4.23	11.32	2.62	3.97		
	22.4	4.03	6.05	2.86	13.49		
	ISDN	4.33	13.95	3.17	25.79		
6	Saline					3.51	0
	0.7					3.61	2.85
	1.4					3.35	−4.56
	2.8					3.96	12.82
	5.6					4.17	18.80
	11.2					4.32	23.08
	22.4						
	ISDN					4.59	30.77
7	Saline	2.42	0	2.80	0		
	0.7						
	1.4	2.77	14.46	3.11	11.07		
	2.8	3.02	24.79	3.39	21.07		
	5.6	2.87	18.60	2.86	2.14		
	11.2	2.69	11.16	3.20	14.29		
	22.4	2.98	23.14	2.74	−2.14		
	ISDN	3.12	28.93	3.29	17.50		

Table 2. *(continued.*

Pt. No.	Substance P Dose (pmol/min)	LAD Diam (mm)	LAD % Diam	LCx Diam (mm)	LCx % Diam	RCA Diam (mm)	RCA % Diam*
8	Saline	2.75	0	2.30	0		
	0.7						
	1.4	2.78	1.09	2.82	22.61		
	2.8	2.77	0.73	2.99	30.00		
	5.6	3.39	23.27	2.81	22.17		
	11.2	3.42	24.36	3.15	36.96		
	22.4	3.62	31.64	3.00	30.04		
	ISDN	3.45	25.45	3.02	31.30		
9	Saline	2.04	0	2.29	0		
	0.7						
	1.4	2.89	41.67	2.73	19.21		
	2.8	2.97	45.59	2.81	22.71		
	5.6	3.02	48.04	2.82	23.14		
	11.2	2.74	34.31	3.41	48.91		
	22.4	2.79	36.76	3.29	43.67		
	ISDN	3.11	52.45	3.09	34.93		
10	Saline	3.10	0	3.45	0		
	0.7						
	1.4	3.48	12.26	3.74	8.41		
	2.8	2.96	−4.52	3.64	5.51		
	5.6	2.75	−11.29	3.36	−2.61		
	11.2	3.19	2.90	3.99	15.65		
	22.4	3.63	17.10	4.22	22.32		
	ISDN	3.68	18.71	4.66	35.07		
11	Saline	2.26	0	3.46	0		
	0.7						
	1.4	2.84	25.66	3.86	11.56		
	2.8	2.90	28.32	3.72	7.51		
	5.6	2.93	29.65	3.87	11.85		
	11.2	2.94	30.09	3.93	13.58		
	22.4	2.93	29.65	4.27	23.41		
	ISDN	3.04	34.51	4.87	40.75		
12	Saline	3.43	0	3.36	0		
	0.7						
	1.4	3.22	−6.12	3.28	−2.38		
	2.8	3.72	8.45	3.31	−1.49		
	5.6	3.70	7.87	3.24	−3.57		
	11.2	3.74	9.04	3.35	−0.29		
	22.4	3.98	16.03	3.59	6.85		
	ISDN	3.79	10.50	4.11	22.32		

*Because control values are expressed as 0, vasoconstriction is expressed as a negative value and vasodilation as a positive value. Diam = diameter; % Diam = percent change in diameter; ISDN = isosorbide dinitrate (2 mg); LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; Pt. = patient; RCA = right coronary artery.

Nellessen et al. (8) demonstrated coronary vasoconstriction in patients after cardiac transplantation as well as in patients without cardiac transplantation or angiographic coronary artery disease. They (8) concluded that acetylcholine may not be a suitable agent for testing endothelial function. In vitro studies (9) have also shown that acetylcholine frequently fails to dilate arterial preparations.

It is probable that the overall effect of acetylcholine depends on the interplay between direct vasoconstriction and endothelium-derived relaxing factor-mediated vasodilation. In view of the widely differing findings obtained from different groups, it appears that acetylcholine is not a specific agent for testing endothelial function. Our findings suggest that substance P may be a more specific marker of endothelium-dependent vasodilation, which produces a greater magnitude of dilation than that achieved with acetylcholine.

Physiologic role of substance P. Substance P is a neuropeptide and immunoreactivity has been demonstrated in the human heart (15). Although its specific role has yet to be

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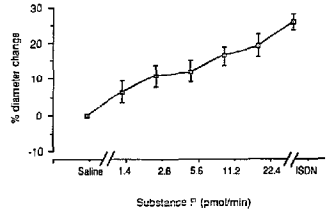


Figure 1. Plot of percent increase in coronary artery diameter (mean ± SEM) in 23 vessel segments after a 2 min infusion of saline solution and substance P in a doubling dosage protocol starting at a dose of 1.4 pmol/min, followed by isosorbide dinitrate (ISDN) at a dose of 1 mg/min for 2 min.

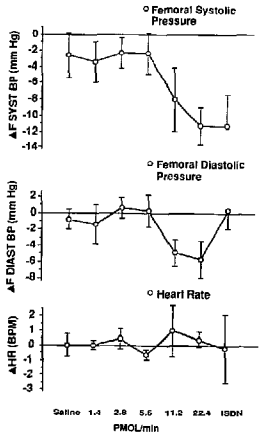
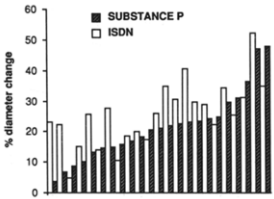


Figure 3. Changes (Δ) recorded in femoral artery systolic pressure (F SYST BP), femoral artery diastolic pressure (F DIAST BP) and heart rate (HR) with increasing concentrations of substance P followed by isosorbide dinitrate (ISDN).

identified, substance P may play a part in the neural regulation of coronary artery tone. Long-term studies after human cardiac transplantation suggest that the donor heart remains functionally (24) and anatomically (25) denervated. We have shown that substance P causes vasodilation in the denervated heart similar to that demonstrated in patients with normally innervated hearts and no evidence of coronary artery disease (17). Denervation, therefore, does not appear to cause significant changes in substance P receptor function, unlike the denervation hypersensitivity demonstrated in response to sympathomimetic amines in the transplanted human heart (26) and in canine preparations (27). In this respect, these results complement the observations of others (28) using animal preparations that hypersensitivity does not occur with other neuropeptides such as calcitonin gene-related peptide. Receptors for substance P have been demonstrated by autoradiographic analysis of the endothelium of the dog carotid (29) and renal (30) artery and human coronary artery (Dashwood MR, personal communication). A recent study (31) demonstrated that substance P is released from the rat hind limb vasculature under conditions of increased blood flow before and after pharmacologic denervation, suggesting that the source of substance P might be the endothelium itself; it was further observed that release of substance P was abolished after removal of endothelium. These studies, in addition to our own findings, suggest that substance P is active mainly at the endothelial level and its action is not affected by denervation despite its demonstration in neural tissue (11–13).

Figure 2. Maximal response (expressed as percent increase in diameter) to intracoronary substance P in 23 vessel segments compared with the response to intracoronary isosorbide dinitrate (ISDN) in the same vessel segments.



Relevance of vasodilator response. The response to substance P in the presence of atherosclerosis has been demonstrated to be preserved in some patients but not in others (32), implying that even in the presence of atherosclerosis, there may be enough functional endothelium to mediate a vasodilator response. It is therefore possible that lack of response to substance P may only be a marker of severe endothelial damage. Bossaller et al. (19), however, showed in isolated preparations of human coronary arteries that the response to substance P in the presence of atherosclerosis is diminished. It is therefore difficult to speculate whether the finding of reduced response to substance P in comparison with that to isosorbide dinitrate in

some of the vessel segments studied represents endothelial dysfunction or the development of undetected coronary artery disease.

Correlation with clinical factors. There was no relation between the magnitude of dilator response to substance P with other factors, such as time after transplantation, donor age, ischemic time of the donor heart and number of rejection episodes. Two patients (Patients 4 and 11) had more than four episodes of rejection early in their postoperative course. One of these patients (Patient 4) had a markedly reduced response to substance P, but the other (Patient 11) had a very powerful vasodilator response. It seems unlikely that there is an association between previous rejection episodes and substance P response. It is not clear, however, what the response would be during the active phase of rejection.

Comparison with isosorbide dinitrate response. Although there was some individual variability in the maximal response to substance P, the mean response was not significantly different from that to isosorbide dinitrate. In most of the vessels studied, substance P achieved maximal or near maximal dilation compared with isosorbide dinitrate. In only two of the vessel segments studied was the response to isosorbide dinitrate substantially greater than that obtained with substance P. In those patients who did not respond well to either isosorbide dinitrate or substance P, it is likely that there is limitation of vasorelaxation, which may in part result from the state of denervation (2).

Conclusions. In cardiac transplant recipients, coronary endothelial function is preserved, as demonstrated by a preserved relaxant response to intracoronary substance P. In patients with no evidence of accelerated coronary artery disease, the degree of response is not affected by the time after transplantation, suggesting that the endothelium remains a functionally active organ in these patients. Substance P appears to be a useful pharmacologic agent for the investigation of endothelial function and is active in very small amounts. Further investigations are necessary to determine endothelial function in those patients who have already developed coronary artery disease to examine the role that endothelium plays in the disease process.

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