Correspondence

Effect of angioplasty and grafting on porcine vascular nerves: a potential neurotropic role for endothelin-1

The most commonly performed procedure for treating coronary artery stenosis is percutaneous transluminal coronary angioplasty (PTCA) and, where the vessel lumen is severely narrowed, coronary artery bypass grafting (CABG). In PTCA, regions of atherosclerotic plaques are disrupted, and the vessel lumen increased by inflating a balloon catheter. In CABG an autologous saphenous vein into coronary artery interposition graft is performed in order to bypass occluded regions of epicardial coronary arteries. Both interventions cause varying degrees of vascular damage and the long-term efficacy of these procedures is limited by a high incidence of neointimal formation and subsequent vascular restenosis (Bach et al. 1994; Bryan & Angelini, 1994).

The endothelium-derived constrictor peptide, endothelin-1 (ET-1) (Yanagisawa et al. 1988), also possesses mitogenic activity on vascular smooth muscle cells (Hirata et al. 1989) and has been suggested as playing a role in atherosclerosis (Dashwood et al. 1993; Zeiher et al. 1994) and intimal hyperplasia (Dashwood et al. 1993; Douglas et al. 1994). We have used 2 experimental models that mimic the occlusive processes observed following CABG and PTCA in order to study the potential role of ET-1 in vascular smooth muscle cell proliferation and neointima formation induced by these methods. As a model of CABG, an autologous saphenous vein into carotid artery interposition graft has been performed that exhibits marked neointima formation within 4 wk (Angelini et al. 1992). PTCA of the left anterior descending coronary artery has also been carried out where pronounced myointimal thickening also occurs within 1 mo (Hata et al. 1995). Apart from a reduction in lumen diameter following these procedures there is a marked development of 'neoadventitia' due to adventitial remodelling. This process is associated with hypercellularity of the adventitial layer involving proliferation of a variety of cell types including fibroblasts (Shi et al. 1996) and an increased density of innervation (McGeachie et al. 1989).

We have studied ET-1 and its receptors and their involvement in neointima formation of porcine vessels. Autologous saphenous vein into carotid artery grafting has been performed in accordance with British Home Office Animal Care regulations as described previously (Angelini et al. 1992; Dashwood et al. 1995, 1997) and, in a seperate series of experiments, coronary angioplasty was carried out on pigs according to the protocol described by Hata et al. (1995). Here we report an unexpected effect of these procedures on the paravascular innervation of the blood vessels studied. The localisation and density of nerves was assessed on ungrafted saphenous veins and vein grafts and injured versus noninjured segments of epicardial coronary artery (following PTCA) by counting the number of nerve bundles (NF200 positive immunostaining) on slide mounted sections from the above vessels (Dashwood et al. 1996). ET-1 binding sites (putative receptors) were also identified on adjacent sections by in vitro autoradiography (Dashwood et al. 1996, 1997).

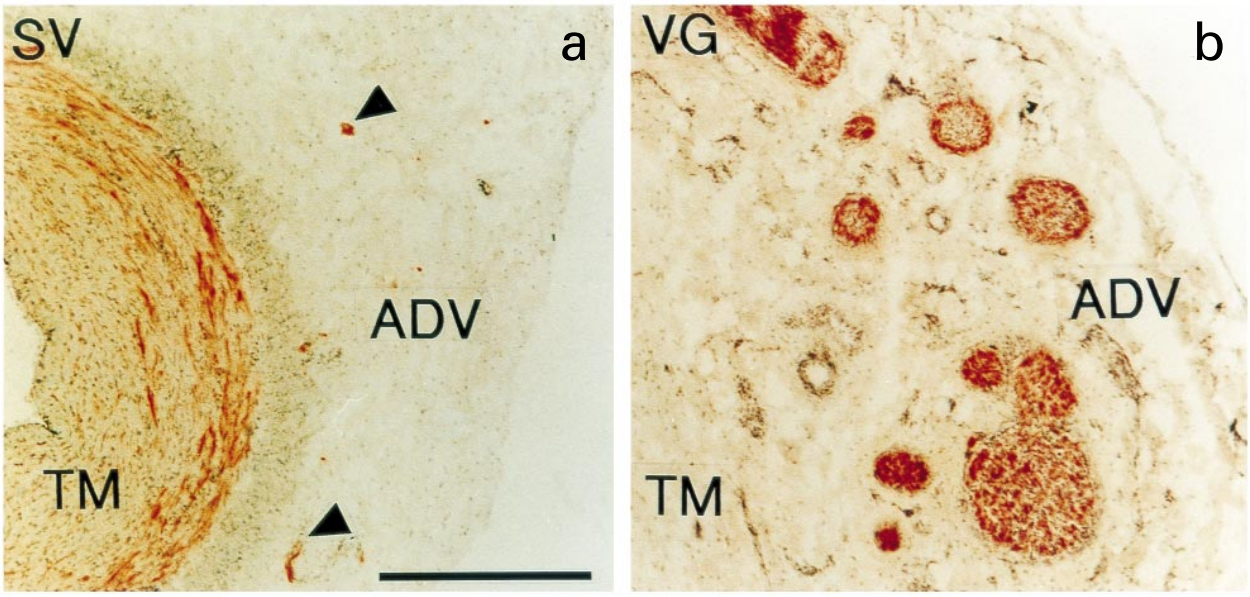

Fig. 1. Innervation of the porcine saphenous vein: effect of grafting. Representative sections of ungrafted porcine saphenous vein (SV, *a*) and grafted vein (VG, *b*). Nerves are identified using NF200 (red stain = positive immunoreactivity). The density of paravascular nerve bundles in the ungrafted vein is low (filled arrowheads), whereas there are abundant paravascular nerves in the grafted vessel. ADV, adventitia; TM, tunica media. Bar, 50 μm.

Table. *Vascular innervation of porcine vessels: effect of PTCA and vein grafting**

	No. of animals	Paravascular nerve trunks	*P*
PTCA			
Noninjured	6	5.4±0.51	0.0142
Injured	6	7.85±0.65	
Vein graft			
Ungrafted vein	5	3.13±0.7	0.0001
Grafted vein	4	13.95±1.3	

* Number of paravascular nerve trunks per vessel section from porcine vessels following PTCA and vein grafting. Mean±S.E.M. of measurements of 4–6 transverse sections of control (noninjured coronary artery, n = 6 animals; ungrafted saphenous vein, n = 5 animals) and experimental (balloon injured coronary artery, n = 6 animals; grafted vein, n = 4 animals) vessels; 2-tailed unpaired *t* test.

Perivascular nerves were identified in vessel sections which were located in close proximity to the external elastic lamina (the medial/adventitial border) and paravascular nerve trunks were also present in the adventitia (Fig. 1). PTCA caused a moderate increase in paravascular innervation since injured segments of porcine left anterior descending coronary artery (LAD) had a higher number of NF200 positive sites than noninjured segments (Table, Fig. 2). A much more pronounced effect was observed in vein grafts. The adventitial innervation of ungrafted saphenous vein was sparse, or not detectable, whereas a dramatic increase in paravascular innervation was observed (see Table) in vein grafts which was located primarily within the outer part of the neoadventitia, some distance from the medial/adventitial border (Fig. 1). The autoradiographic studies revealed dense [^{125}I]ET-1 binding to the tunica media of all vessels as well as to the neointima of vein grafts (Dashwood et al. 1995, 1997) and balloon-injured coronary arteries (M. Kirchengast, K. Muenter & M. R. Dashwood,

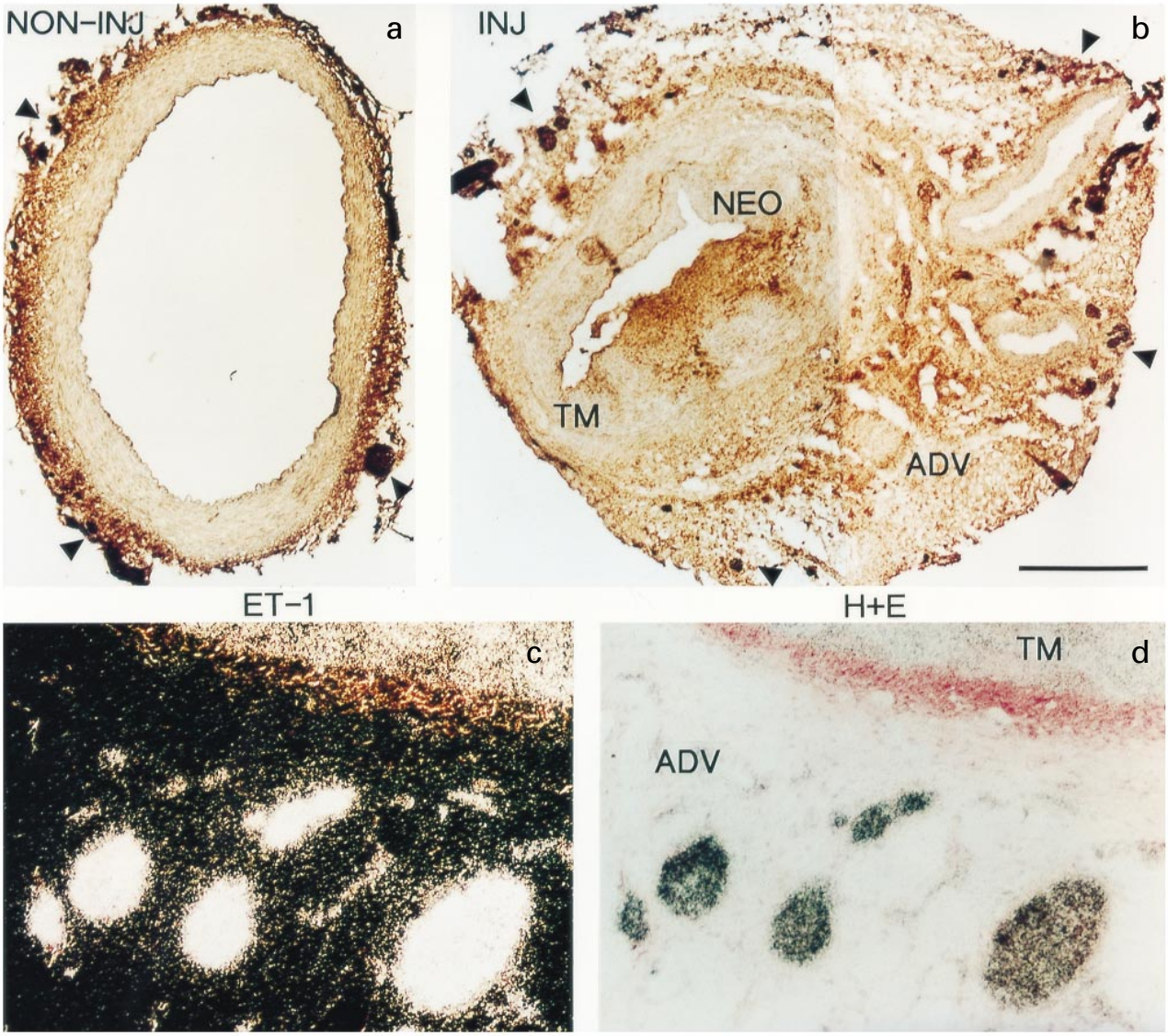

Fig. 2. Innervation of the porcine epicardial, coronary artery: effect of PTCA and location of ET-1 receptors. Vascular nerves are identified on sections of noninjured (*a*) and injured (*b*) porcine epicardial artery (NF200-positive brown staining, arrowheads). Note the neoadventitia on the photomontage of the injured segment. (*c*) ET-1 binding to paravascular nerves of injured vessel segment. Dark-field illumination, where [^{125}I]ET-1 binding sites are evident as white grain accumulation on a black background. (*d*) Haematoxylin and eosin stained tissue underlies the autoradiograph. TM, tunica media; ADV, adventitia; NEO, neointima. Bar, 250 μm in *a*, *b*, 50 μm in *c*, *d*.

unpublished results). There was also dense binding to the paravascular nerve trunks (Fig. 2).

Neointima formation and vessel occlusion of vein grafts and restenosis following PTCA is well established. A number of experimental models have been developed to study vascular smooth muscle cell migration, deposition of extracellular matrix proteins and other events underlying neointima formation. However, data regarding the effect of these procedures on the adventitia are limited. Following balloon angioplasty, a certain degree of adventitial remodelling occurs, where proliferation of fibroblasts and modulation of their phenotype has been described that is associated with a thickened adventitial layer (Shi et al. 1996). There is also some evidence of increased vascular innervation following balloon catheter injury of the rat carotid artery (Milner et al. 1997) as well as in experimental vein grafting in this species (Waris et al. 1984). The results presented in our study suggest that vascular injury may influence the paravascular innervation of blood vessels in certain circumstances. It would appear that the degree of reinnervation is associated with the severity of vascular injury since balloon angioplasty ('moderate injury') had a less pronounced effect on nerve density than vein grafting (where the vessel is essentially denervated; 'severe injury'). ET-1 binding to paravascular nerves of human and porcine coronary artery (Power et al. 1989, Dashwood et al. 1996) and peripheral nerve has been described (Dashwood & Thomas, 1997). The presence of ET-1 receptors at regions of 'neoinnervation' of porcine vein grafts and their association with the increased number of paravascular nerves of coronary arteries following balloon injury suggest that this peptide, or other unidentified neurokine(s), may have neurotropic and/or neurotrophic role(s) in vascular injury. There is recent evidence that ET-1 and its receptors are associated with a number of adventitial structures of diseased or injured blood vessels (e.g. neovascularisation and inflammatory cells; Dashwood et al. 1993, 1995, 1997). It is therefore possible that, following vascular injury, local tissue levels of ET-1 are raised (released from adventitial monocytes, fibroblasts etc), and that under such conditions this peptide possesses neurotropic/neurotrophic activity and promotes vascular reinnervation.

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