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ANGIOTENSIN II RECEPTORS IN RABBIT VASCULAR GRAFTS

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New Zealand White rabbits were used as a model of autologous venous bypass grafting. A section of jugular vein was sutured into the divided common carotid artery. The animals were euthanized one month after surgery. At that time histopathology showed myointimal thickening of the graft segments. Angiotensin II receptor assays showed that the AT_1 receptor was present in the grafts, concentrated in the intima. These localized receptors were shown to be similar to the prototypic vascular AT_1 receptor through the use of the specific antagonist DuP 753.

The use of autologous veins for arterial repair has been noted in the literature since the early 1950's (1), but when autologous venous material is used to bypass arterial occlusions due to atherosclerosis, the long-term failure rate is quite high (2,3). Angiographic studies, biopsies done at the time of re-operation for restenosis, and post-mortem findings indicate that the venous graft material develops, lesions comparable to those seen with the long-term development of atherosclerosis within a compressed time frame (4,5,6). The grafts are susceptible to the full array of cardiovascular risk factors and are also in the state of adaptation, having gone from the low pressure venous system to the higher pressure arterial system. It has been suggested that some of the "arterialisation" seen in graft material is simply a response to pressure change. A number of studies have considered the role of the renin-angiotensin system in atherosclerosis and the use of angiotensin-converting enzyme (ACE) inhibitors in the suppression of myointimal proliferation after vascular injury. The use of ACE inhibitors has been shown to decrease the development of atherosclerotic lesions in WHHL rabbits (7) and non-human primates (8) and to decrease the myointimal proliferation seen following balloon angioplasty (9). It is not clear whether angiotensin II (AII) has a direct effect on the cells of the myointima or whether it modulates the effects of other cytostimulants.

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In this study we considered the possibility of a localized AII response and demonstrate here the presence of AII receptors in the intima of the venous grafts. The localized AII receptors are shown to be similar to the prototypic vascular AII receptor (AT_1) through the use of the specific antagonist DuP 753 (10).

METHODS

Ten male New Zealand White rabbits weighing from 2.5 to 3.3 kg were maintained on a normal light cycle and fed a standard chow diet. The surgery followed the procedures of Zwolak et al.(11) with some modifications.

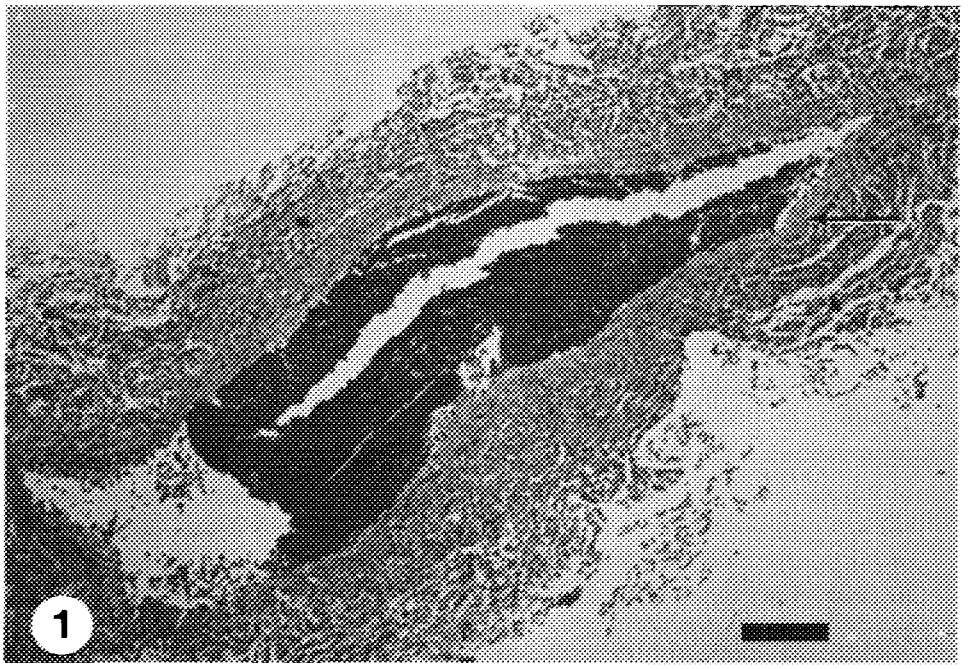
Anesthesia was induced with intramuscular acepromazine (0.3 - 0.4 mg/kg) and ketamine (40 mg/kg). Maintenance of anesthesia was by inhalant isoflurane. The right common carotid artery and the jugular vein were exposed by a ventral midline incision of the neck, then dissected free. After intravenous administration of heparin (1000 U) a 3.0-cm segment of the jugular vein was sutured into the carotid artery using simple interrupted sutures of 10-0 Prolene. The animals were maintained in an AALAC accredited facility in accordance with the guide-lines of the Animal Welfare Act.

Four weeks after the surgical procedure, the rabbits were anesthetized with the induction accomplished by intramuscular acepromazine and ketamine and maintained by inhalant Isoflurane. A 22 gauge Angiocath was introduced into the abdominal aorta and the vascular system flushed with lactated Ringer's solution. The graft and several millimeters of carotid adjacent to the anastomoses were then excised and placed immediately into cold physiologic saline. A corresponding section of the unoperated vein was taken for comparison purposes. Sections of both the unoperated vein and the graft were preserved in formalin for histopathology. Graft patency was >80%.

Autoradiography. - Fresh tissue was frozen on powdered dry ice and stored at -70°C until used. Sections were cut at 15 microns and thaw-mounted onto gelatin stubbed slides. Autoradiography was performed according to the methods of Chiu et al. (10). Slides were placed under Kodak XAR-5 radiograph film and exposed for 7 days before processing the film to produce the autoradiograms. Image analysis of the films was achieved by computerized digitization of the autoradiographic images using the DUMAS system (Drexel University, Philadelphia, PA).

RESULTS

Histopathology of the sections used for the AII autoradiography showed myointimal proliferation in each of the observed graft sections relative to the unoperated control sections of jugular vein (Figures 1, 2). These data are consistent with previous findings of myointimal proliferation in this model (11).



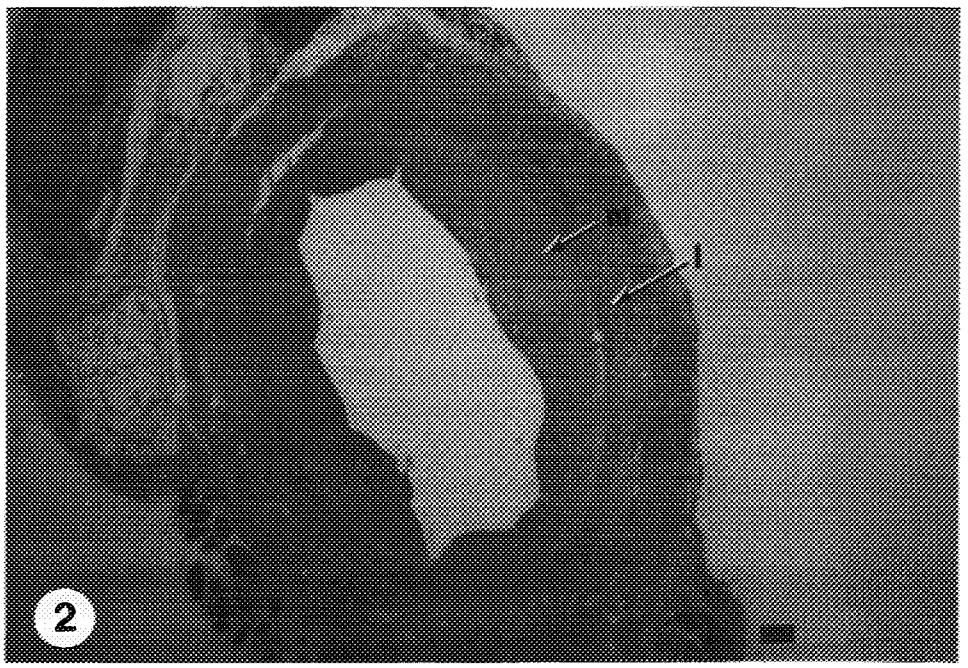


Figure 1. Cross section of unoperated (normal) jugular vein, stained with hematoxylin and eosin. There is a relatively thin intima (arrow) and overall wall thickness. Red blood cells are present in the lumen. Scale bar represents 100 microns.

Figure 2. Hematoxylin and eosin cross section of the jugular vein graft one month after it was implanted in the carotid artery. Scale bar represents 100 microns. Relative to the unoperated section shown in Figure 1, the overall thickening of the vessel wall may be noted. There is an increase in thickness and cellularity of the myointimal layer (m). The internal elastic lamina is indicated at I.

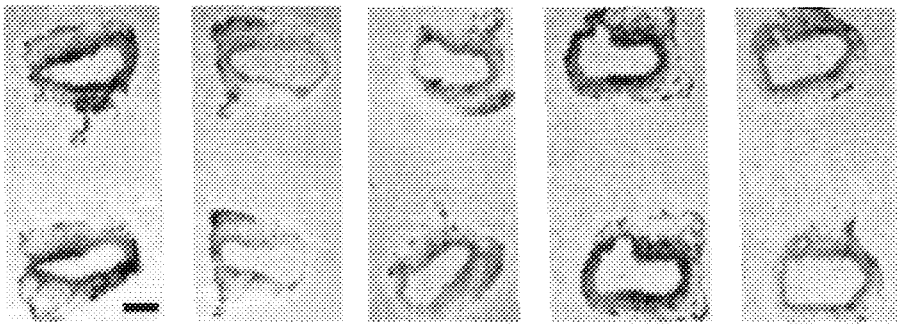


Figure 3. (A, B, C, D, and E) Cross sections of jugular vein graft one month after implantation into the carotid artery. The images presented here are the autoradiograms from the AII receptor assay as viewed on the image analyzer. Scale bar in A represents 1 mm. A: Total binding in the AII receptor assay. Binding is primarily in the intima and myointima. B: Non-specific binding in the presence of 3 μM AII. The intensity of binding has been greatly decreased, showing that total binding is greater than non-specific. C: Graft cross section treated with DuP 753 (10 μM), a specific antagonist for the AT_1 receptor subtype. Intensity of binding is greatly decreased. D: Graft cross section treated with PD123177, an antagonist selective for the AT_2 receptor subtype. There is no detectable change in the intensity of binding. E: Graft cross section treated with saralasin (1μM), a peptide that blocks both AT_1 and AT_2 receptor subtypes. Binding is reduced to the non-specific level.

Neither the sections of artery nor the unoperated vein showed evidence of AII binding (data not shown). In the sections of graft, however, total binding was greater than nonspecific, and was concentrated in the intima (Figure 3). There was non-uniform distribution of binding sites per section and some variability of intensity and distribution throughout serial sections. Three compounds were used to identify the subtypes of AII receptors involved. DuP 753, a nonpeptide AII receptor antagonist specific for the AT_1 receptor subtype (10), greatly decreased the intensity of specific AII binding when applied at a concentration of 10 μM. PD123177, another nonpeptide AII receptor antagonist selective for the AT_2 receptor subtype (10), produced no apparent change in intensity of binding at 10 μM. At a concentration of 1 μM, saralasin, a peptide analog of AII which blocks both AT_1 and AT_2 subtypes, essentially eliminated specific binding over the intima. This indicates that the predominance of receptors expressed in vein graft sections are of the AT_1 subtype.

DISCUSSION

The blood vessel wall has been shown to respond to a variety of stimuli that may very generally be categorized as denuding versus non-denuding injury. Changes in blood flow dynamics, such as hypertension, or changes in blood

viscosity due to hyperlipidemia may be classified as non-denuding injuries. That is, they are stimuli that do not acutely remove or damage the endothelial cell layer yet cause pathologic changes in the vessel wall. Various researchers have shown that hypertension produces marked effects on the endothelium. Among the changes noted have been increased endothelial layer permeability, increased adherence of white blood cells to the endothelium, and a decrease in endothelium-modulated relaxation of the arterial wall (13,14). Haudenschild et al. (14) showed changes in the arterial media in response to hypertension. The change in thickness appeared to be due to increases in both smooth muscle cellularity and extracellular matrix. When there is denuding injury to the vessel wall, the response is a migration of smooth muscle cells from the media to intima, accompanied by proliferation of these cells and the extracellular matrix. Powell et al. hypothesized a local angiotensin system mediating response to vascular injury (9). They reported that continuous administration of the ACE inhibitor cilazapril to rats decreased the proliferative response to balloon catheter endothelial injury and that the reduction in proliferative response appeared to be due to both fewer smooth muscle cells and less connective tissue formation.

The effect of the ACE inhibitors on atherosclerosis development has also been demonstrated in rabbits and non-human primates. Watanabe heritable hyperlipidemic rabbits (WHHL) dosed orally for nine months with captopril showed a decrease in the area of total aortic lesions of atherosclerosis compared to untreated cohorts (7). Aberg and Ferrer (8) showed a lessening of atherosclerotic involvement in Cynomologus monkeys treated with captopril for six months. McCann et al. (12) in their study of the changes in autologous vascular grafts in non-human primates, showed changes in intimal morphology and lipid composition related to material used for the graft and the blood lipid profile, but not totally dependent upon those factors. Their conclusion was that the fact of transplantation was a greater factor influencing the venous graft changes than either the presence or absence of hyperlipidemia or the type of tissue used for grafting. This is supported by our study in which the changes experienced by the transplanted venous graft concurrent with the change in anatomical location were sufficient to induce the presence of receptors.

Our demonstration of the consistent presence of AII receptors on the intima of autologous venous grafts may help to explain the observed effect of ACE inhibitors. They give support to various observations of AII causing hypertrophy of smooth muscle cells in culture (15) which may now be correlated with in vivo expression of AII receptors. The question still remains as to whether AII has a direct effect on the myointima or modulates other vasoactive factors. It is also unclear as to whether the changes in blood pressure influence the permeability of the endothelial cell layer and

possibly the entire vascular wall through an AII-mediated response. Studies with the autologous vein graft model used here may lead to answers to these questions.

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