Lanthanum Staining of Coronary Microvascular Endothelium: Effects of Ischemia Reperfusion, Propranolol, and Atenolol

D. W. Haack, L. R. Bush, M. Shlafer, and B. R. Lucchesi

Department of Physiology, University of Arizona College of Medicine, Tucson, Arizona 85724, and Department of Pharmacology and Upjohn Center for Clinical Pharmacology, University of Michigan, Ann Arbor, Michigan 48109

Received June 24, 1980

Cat isolated hearts were perfused via the aorta with normothermic arterial blood from donor cats. After 1 hr of equilibration, dl-propranolol (1.9 mg/kg), atenolol (1.65 mg/kg), or physiological saline solution was infused via the aortic cannula. The hearts were made globally ischemic for 1 hr and reperfused for 1 hr. Hearts given saline but not made ischemic, and hearts from blood-donor cats served as controls. The hearts were flushed with physiological saline for 2 min, then perfused with cacodylate-buffered glutaraldehyde containing 1% LaCl3. Samples of left ventricle were postfixed in osmium and prepared for electron microscopy. Microvessels in nonischemic tissues had heavy La3+ staining on luminal surfaces of endothelial cells. Many plasmalemmal vesicles along luminal surfaces of endothelial cells were filled with La3+. Several vesicles appeared to open onto both surfaces thus forming channels through the endothelium. Lanthanum penetrated into, and occasionally through, interendothelial junctions. Endothelial cells lining vessels in ischemic myocardium were swollen, had pale cytoplasm, and showed little La3+ on the luminal surfaces. Few plasmalemmal vesicles were present and the mitochondria contained deposits of La3+. Extravascular spaces were distended but interendothelial junctions seemed to be intact. Lanthanum staining and morphology of endothelial cells in hearts treated with propranolol or atenolol were very similar to nonischemic myocardium. The data suggest that the β-blocking agents, propranolol and atenolol, maintain the integrity of coronary vascular endothelium during ischemia.

INTRODUCTION

Much of the research on myocardial ischemia has been aimed at preservation of myocardial function upon restoration of an adequate blood supply to the affected area. The structure and function of the myocardium after ischemia and reperfusion have been studied by a number of investigators (Althaus et al., 1977; Apstein et al., 1978; Bulkley and Hutchins, 1977; Burton et al., 1977; Bush et al., 1980b, c; Gaasch et al., 1978; Jennings and Ganote, 1974, 1976). However, little attention has been directed toward the structure of the myocardial vasculature. Ischemia has been reported to cause swelling of endothelial cells in the coronary microcirculation and to increase endothelial permeability (Poche, 1969; Kloner et al., 1974, 1977; Meneely, 1974; Gavin et al., 1978). Preservation of microvascular integrity is central to the maintenance of myocardial function after ischemic episodes for several reasons. First, an intact microvasculature would allow an
adequate blood supply to the affected tissue to be reestablished easily. Second, preservation of microvascular integrity would protect the microenvironment within which the myocardial cells exist. This would directly support the function of the myocardial cells. Finally, an intact microvasculature would allow for better delivery of cardioprotective drugs to the affected tissues.

Lanthanum has been used to study the effects of cations on cellular function (Weiss, 1974; Dunnett et al., 1978; Mela, 1968; Langer and Frank, 1974), the structure of intercellular junctions (Revel and Karnovsky, 1967), the structure and composition of the external cell surface (Doggenweiler and Frenk, 1965; Frank et al., 1977; Lesseps, 1967; Martinez-Palomino et al., 1973; Shea, 1971), and the permeability of blood vessels (Huttner et al., 1975; Jokelainen et al., 1976). Recently lanthanum has been used as a probe of myocardial cell membrane integrity after ischemic episodes (Burton et al., 1977; Hoffstein et al., 1975) and as a test of the ability of various pharmacologic agents to protect myocardial cells from the effects of ischemia and reperfusion (Burton et al., 1980; Bush et al., 1980a, b). The β-adrenergic antagonists, propranolol and atenolol, have been shown to protect the structure and function of myocardial cells during ischemia and reperfusion (Reimer et al., 1976; Bush et al., 1980a, b; Shlafer et al., 1980) but the mechanism of this protection is not known. In addition, the significance of endothelial disruption in the course of several pathologic processes recently has been reviewed (Thorgeirsson and Robertson, 1978). However, the role of the coronary microvascular endothelium in the damage produced by myocardial ischemia and reperfusion is not known. Therefore, the purpose of the present study was to use ionic lanthanum to probe the integrity of endothelial cells lining the coronary microvasculature after global cardiac ischemia and reperfusion with or without pretreatment with β adrenergic antagonists. A preliminary report of this work has been presented (Haack et al., 1980).

MATERIALS AND METHODS

Isolated heart preparation. The cat isolated heart preparation used in this study was identical to that described by Vogel et al. (1979). Hearts were taken from 1- to 2-kg cats which had been anesthetized with pentobarbital (30 mg/kg, ip). The hearts were perfused via the aorta with arterial blood drawn from 2.5- to 3.5-kg cats which had been anesthetized with dial urethane (0.7 ml/kg, ip). The dialurethane was prepared as a solution containing 100 mg/ml allobarbital, 400 mg/ml urethane, and 400 mg/ml monoethyl urea. Perfusion pressure to the isolated hearts was maintained between 70–90 mm Hg by means of a roller pump. Left ventricular developed pressure was maintained at 150 mm Hg by adjusting the volume of a saline-filled balloon that had been placed in the left ventricle. The hearts were electrically paced at 150 beats per minute. Coronary effluent blood collected from the isolated heart by means of a cannula in the pulmonary artery was returned to the blood-donor cat via a cannula in its jugular vein. Isolated hearts were allowed to equilibrate for 1 hr.

Experimental protocol. After the equilibration period dl-propranolol (1.9 mg/kg donor-cat body wt), atenolol (1.65 mg/kg donor-cat body wt), or saline (0.15 ml/min/10 min) was injected into the aortic cannula of the isolated heart by a
Harvard infusion pump. The latter group of hearts served as nontreated ischemic controls. The hearts were then exposed to 1 hr of normothermic ischemia by turning off the perfusion pump. Hearts that were given saline but not made ischemic, and hearts from the blood-donor cats, served as normal controls. Following the ischemic episode the hearts were reperfused for 1 hr. There were at least five hearts per group. One hour of global normothermic ischemia followed by an hour of reperfusion causes nearly a 60% decline in contractility and compliance (Vogel et al., 1979; Apstein et al., 1978). In similar models normothermic ischemia also has been shown to produce significant disruptions of myocardial function and ultrastructure (Apstein et al., 1977; Gillette et al., 1979).

Electron microscopy. After the reperfusion period two hearts were selected at random from each group. Each heart was given a code number to obscure the identity of the group from which it was obtained. The numbers were not decoded until data collection had been completed. The hearts were perfused with oxygenated physiological saline, 30°, for 2 min. During this time the intraventricular diastolic pressure was adjusted to 10 mm Hg. The saline solution contained (in...
Nonischemic control. This is an oblique section of a capillary. Lanthanum has uniformly stained the luminal surface (LS) and has entered the perivascular space (PS). The presence of La\(^{3+}\) in the vesicles indicates that they open onto the cell surface either directly (arrows) or via complex interconnections (*). 26,300×.

mM): NaCl, 140; KCl, 5; MgCl\(_2\), 1.2; glucose, 10; CaCl\(_2\), 1.8; and Tris buffer, 5, pH 7.4. Then the hearts were perfused with 0.1 M sodium cacodylate containing 2.5% glutaraldehyde and 1% LaCl\(_3\). The pH of the fixative was 7.4 and the temperature was 25°. Perfusion pressure was maintained between 65 and 75 mm Hg during the saline rinse and fixation. Fixative was perfused through the hearts for 3 min and then the hearts were immersed in fixative. The hearts were weighed and tissue samples were obtained from left ventricular papillary muscles and subendocardium. Tissue samples were cut into cubes 1–2 mm on a side and reimmersed in fixative at 25°. Total fixation time was 2 hr. The tissue samples were stored overnight at 4° in 0.1 M cacodylate containing 8% sucrose, pH 7.4, and then postfixed in cacodylate-buffered 1% osmium tetroxide for 1 hr. After dehydration in ethanol and propylene oxide, and embedding in Epon, the tissue blocks were mounted on Epon posts and sectioned with an LKB-Huxley ultramicrotome. Thick sections mounted on glass slides and stained with toluidine blue were examined with a light microscope to determine the orientation of the tissue. The tissue blocks were oriented so that capillaries would be sectioned transversely rather than longitudinally, because identification of capillaries is easier when the
vessels have been cut in cross section. At least three blocks from each tissue sample were sectioned for electron microscopy. Thin sections were mounted on copper grids and examined with a Siemens 101 electron microscope. Both nonstained sections and sections that had been stained with uranyl acetate and lead citrate were studied. No differences in lanthanum distribution were noted between stained and unstained sections.

RESULTS

The endothelial cells surrounding capillaries in nonischemic control hearts and in hearts from blood-donor cats (Figs. 1–4) were very similar in morphology to capillaries described by others (Fawcett and McNutt, 1969; Sherf et al., 1977; Rhodin, 1974; Simionescu and Simionescu, 1977). The endothelium formed a layer of uniform thickness, except in the region of the nucleus, around an ovoid-to-circular lumen. One or two interendothelial junctions were present in each capillary. The endothelial cells contained a small number of mitochondrial profiles and many plasmalemmal vesicles. Each capillary was surrounded by a basal lamina. Occasionally the perivascular space was so compact that the basal laminae of the endothelial and myocardial cells appeared to be fused (Fig. 2).
The most striking feature of these vessels was the presence of a filamentous coat on the luminal surfaces of the endothelial cells (Figs. 1–4). This coat was especially prominent due to its staining by lanthanum. Lanthanum also was present on the basal laminae in the perivascular spaces. In many vessels lanthanum was present in the endothelial plasmalemmal vesicles (Figs. 2–4) as well as the interendothelial junctions (Figs. 1 and 3). However, it was not possible to determine whether lanthanum had reached the perivascular spaces via interendothelial junctions or channels through the endothelial cells formed by plasmalemmal vesicles (Fig. 4). No lanthanum was observed within the endothelial cells of capillaries in control hearts.

In contrast to the control capillaries, microvessels in hearts that had been ischemic for 1 hr and reperfused for another hour were poorly preserved (Figs. 5 and 6). The most striking difference between control and ischemic capillaries was the absence of lanthanum staining from the luminal surfaces of capillaries in the ischemic hearts. In addition, endothelial cells in ischemic hearts were swollen, had pale-staining cytoplasm, and indistinct or disrupted plasma membranes. Some endothelial cells contained few plasmalemmal vesicles (Fig. 6) whereas others were replete with vesicles (Fig. 5). Dense accumulations of lanthanum were located...
FIG. 5. Ischemia reperfusion, no drugs. The most striking feature of this micrograph is the lack of either La²⁺ or a filamentous coat on the luminal surface (LS) of this vessel. Lanthanum is present in the perivascular connective tissue (arrows). Both cells have indistinct or disrupted membranes and one cell (*) appears to be swollen. 21,500×.

In mitochondria of many endothelial cells (Fig. 6). Lanthanum staining of the basal laminae was not markedly increased but the perivascular spaces were distended. In spite of extensive disruption of the endothelial cells the interendothelial junctions appeared to be structurally intact.

The morphology of capillaries in hearts that had been treated with propranolol or atenolol was very similar to that of control capillaries (Figs. 7-10). The filamentous endocapillary coat was present and heavily stained with lanthanum. The endothelial cells were thin, well preserved and filled with plasmalemmal vesicles. The interendothelial junctions contained lanthanum but did not differ morphologically from those in capillaries from nonischemic control hearts. However, the perivascular spaces were somewhat wider than those observed in control tissues. Pretreatment with propranolol or atenolol attenuated the deleterious effects of ischemia and reperfusion on the myocardial microvasculature. There were no apparent differences between the hearts treated with propranolol and those treated with atenolol.
FIG. 6. Ischemia reperfusion, no drugs. The amount of La$^{3+}$ on the luminal surface of this vessel is markedly reduced but La$^{3+}$ is present in the interendothelial junction (IJ). Striking La$^{3+}$ accumulations are apparent in the mitochondria (M) of the endothelial cell. Lanthanum is also seen in the perivascular space. The endothelial cells appear to have been thoroughly disrupted. 16,800×.

DISCUSSION

The data presented in this report demonstrate that the luminal surfaces of myocardial capillary endothelial cells were covered by a fine filamentous layer which normally stains heavily with ionic lanthanum, but that after a period of ischemia and reperfusion this endocapillary layer was lost. In addition, the endothelial cells and surrounding myocardial cells were structurally disrupted after an episode of ischemia and reperfusion. However, treatment of hearts with propranolol or atenolol prior to the period of ischemia and reperfusion prevented the loss of the endocapillary layer and attenuated the damage to both endothelial and myocardial cells (Bush et al., 1980a, b). The β-blocking drugs also preserve myocardial cell function (Shlafer et al., 1980). These data confirm and extend observations made by Kloner and his co-workers (1977) concerning the ability of propranolol to protect the coronary microvasculature against the effects of ischemia followed by reperfusion. Although the mechanism of this protection is not
The filamentous material on the luminal surface of this capillary has been well preserved and lightly stained with La$^{3+}$. Lanthanum is present in the interendothelial junction (IJ) and a few vesicles (arrows) but little is present in the perivascular space. 24,400 x.

known, the data suggest that a relationship may exist between preservation of the endocapillary layer and maintenance of endothelial cell integrity.

The presence of a specific coat on the luminal surface of endothelial cells was postulated as early as 1947 by Chambers and Zweifach to explain some characteristics of capillary permeability. In 1965, Luft presented the first morphological evidence of such a coat and suggested that it consisted of acid mucopolysaccharides, indicating that the coat was not produced by accretion of plasma proteins onto the surfaces of the endothelial cells (Luft, 1966). More recently cationized ferritin has been used to confirm the existence of the luminal surface coat and to show that approximately half of the anionic sites in the coat were due to the presence of sialic acid residues (Skutelsky and Danon, 1976). Finally, the well-documented staining of cell surfaces by lanthanum (Doggenweiler and Frenk, 1965; Shea, 1971; Langer and Frank, 1972; Martinez-Palomo et al., 1973) supports the concept that the lanthanum staining observed in the present study and in the work of Weihe et al. (1977) is due to interaction of lanthanum with anionic sites on the endothelial cell luminal surface.
The function of the endothelial luminal coat, or endocapillary layer, is not completely known (Thorgeirsson and Robertson, 1978) but Becker and Harpel (1976) have proposed that it may play a role in maintaining endothelial cell integrity. This endothelial coat is apparently very fragile and consequently is lost during routine preparation of tissues for electron microscopy (Luft, 1966). Therefore the endocapillary layer is rarely seen in electron micrographs. The presence of lanthanum in perfusates and fixatives seems to stabilize this layer and to increase its electron opacity. The apparent lability of the endocapillary layer and the proposed role of the layer in protecting the endothelium from blood-borne elements suggests that loss of the luminal coat may be an early event in the course of endothelial damage produced by ischemia and reperfusion. Conformation of this hypothesis awaits a careful examination of the sequence of endothelial cell changes induced by ischemia and reperfusion.

Restoration of adequate blood flow to an ischemic area of myocardium is essential for support of compromised cells and repair of damaged tissue. However, reperfusion of the ischemic myocardium may not change the course of the pathologic process (Althaus et al., 1977) and may produce further damage.
FIG. 9. Ischemia reperfusion, atenolol. As in capillaries from propranolol-treated hearts the endothelium in this capillary has been well preserved and its luminal surface (LS) has been heavily stained with La$^{3+}$. Lanthanum has penetrated into the interendothelial junction (IJ) and a few vesicles (arrows) but little is present in the perivascular space. 17,150×.

(Bulkley and Hutchins, 1977; Jennings and Ganote, 1976; Vatner et al., 1978). Upon restoration of blood flow to an ischemic area there is an abnormal retention of fluid and ions (Willerson et al., 1977; Whalen et al., 1974) as well as an increase in the permeability of myocardial cell membranes (Burton et al., 1977; Bush et al., 1980a, b). Morphologically the effects of ischemia followed by reperfusion are expressed as swelling of the myocardial cells, swelling and eventual disruption of mitochondria, accumulation of lipid- and calcium-rich granules in mitochondrial matrices, and condensation of chromatin within myocardial cell nuclei (Jennings and Ganote, 1974, 1976; Kloner et al., 1974; Whalen et al., 1974).

The edema observed in the myocardium might be caused by increased permeability of the coronary microvasculature, since a number of insults, including anoxia, reportedly cause opening of interendothelial junctions (Constantinides and Robinson, 1969; Ryan and Majno, 1977), and several markers of vascular permeability have been found to move into injured myocardium (Meneely, 1974; Boutet et al., 1976; Kloner et al., 1977). However, few, if any, of the interendothelial junctions observed in the present study were disrupted. The junctions contained lanthanum but appeared to be structurally intact. The data support previous
FIG. 10. Ischemia reperfusion, atenolol. The luminal surface of this capillary has bound a great deal of La$^{3+}$. The perivascular space is compact and both the endothelial and myocardial cells appear to be intact. Several lanthanum-filled vesicles appear to bridge the endothelial cytoplasm (arrows) but very little lanthanum has entered the perivascular space. 27,000×.

studies that also reported intact interendothelial junction in spite of ischemia (Armiger and Gavin, 1975; Gavin et al., 1978). There are two explanations for these differences. First, the increased permeation of the vasculature may occur by way of the disrupted endothelial cells. Second, different segments of the microvasculature differ in their permeability characteristics (Simionescu et al., 1975), and although precise identification of the various microvascular segments is difficult (Simionescu et al., 1978; Johansson, 1979), the present study focused on capillaries rather than the more permeable postcapillary venules. Therefore “leaky” junctions would not have been found.

The β-adrenergic antagonist propranolol has been shown to reduce the size of myocardial infarcts (Maroko et al., 1972; Reimer et al., 1976) and to protect the coronary microvasculature from the effects of ischemia and reperfusion (Kloner et al., 1977). However, the mechanism of the protection is not known. To gain further insight into this problem the β-adrenergic antagonists, propranolol and atenolol, were compared for their relative actions in preserving the integrity of the myocardial microvasculature in the face of ischemia and reperfusion. Propranolol possesses negative ionotropic and chronotropic effects that are said to be due to
"membrane stabilization" (Frishman and Silverman, 1979). Atenolol at the dose used in our experiments possesses equal β-adrenergic blocking activity, but it lacks propranolol's membrane effects (Barrett, 1977; Frishman and Silverman, 1979; Johansson, 1979). Nevertheless, these agents were equally effective in preventing the ultrastructural changes observed in capillaries from nontreated, ischemic-reperfused hearts. Therefore protection appeared to be provided by β-adrenergic blockade, or at least the result of action other than nonspecific "membrane stabilization."

ACKNOWLEDGMENTS

Supported by NIH Grant HL-19782-03 and grants from the Michigan Heart Association and the American Heart Association.

REFERENCES


LANTHANUM STAINING OF ENDOTHELIAL CELLS


HAACK ET AL.


