Nonuniformity of Endothelial Constitutive Nitric Oxide Synthase Distribution in Cardiac Endothelium

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Abstract—Endocardial endothelium and endothelium of coronary vessels produce NO. Histochemical methods have suggested that coronary arterial endothelial cells contain more endothelial constitutive NO synthase (ecNOS) than does coronary venous endothelium. We have further investigated the distribution of ecNOS in cardiac endothelium using immunofluorescence and en face confocal microscopy of rat heart. In endocardial endothelium, confocal microscopy revealed distinct ecNOS labeling of peripheral cell borders, cytoplasmic labeling, and labeling of the Golgi complexes. Labeling of the cell borders and of the Golgi complexes was confirmed by double staining for ecNOS and for platelet and endothelial cell adhesion molecule or Golgi 58k protein, respectively. Cytoplasmic labeling was strongest in coronary arterial endothelium. The size of the ecNOS-labeled Golgi complexes decreased from coronary arterial endothelial cells (8.63±0.39 μm², mean±SE of 5 rats) to endocardial endothelium (7.07±0.61 μm²) and to coronary venous endothelium (3.65±0.20 μm²). In addition, pixel intensity of ecNOS labeling was higher in arterial endothelial cells than in venous endothelial cells. Endothelium of myocardial capillaries also contained small ecNOS-labeled Golgi complexes. No correlation was observed between endothelial cell surface area and Golgi complex size. Caveolin-1 labeling was strongest in capillaries and did not coincide completely with ecNOS labeling in endocardial and venous endothelium. These results suggest that endocardial and coronary arterial endothelium in the rat have a higher synthetic activity and might express more ecNOS than is expressed by cardiac venous and capillary endothelium. The observed heterogeneity in ecNOS distribution might be related to the specific mecanochemical environment and function of each endothelial compartment. (Circ Res. 1998;82:195-203.)

Key Words: constitutive nitric oxide synthase • endocardial endothelium • coronary endothelium • Golgi complex • caveolin

Nitric oxide is a widespread biological mediator that is implicated in various physiological and pathophysiological processes. In the heart, NO modulates not only coronary flow but also myocardial performance. This latter feature has been shown in several species, including humans. Whereas higher concentrations of NO produce a negative inotropic effect, at low concentrations it can induce a distinct positive inotropic effect. In physiological conditions, NO is synthesized from L-arginine by constitutive NOS. The two known isoforms of NOS type III) in cardiac endothelium. Compared with ecNOS labeling in the endothelium of coronary vessels, a modest ecNOS labeling has also been observed in cardiac myocytes, where it appears to be associated with caveolin-3, a muscle-specific isoform of a coat protein of caveole.

In ECs, ecNOS is associated primarily with the particulate fraction, in particular with the Golgi complex, and with domains of the plasma membrane, the caveole. The Golgi complex (also called Golgi apparatus) consists of one or more stacks of cisternae surrounded by vesicles. The Golgi complex is the site of biosynthesis of glycolipids and of sugar moieties of glycoproteins. The processed proteins, lipids, and polysaccharides are either directly secreted or sorted in the Golgi complex and transported to other cell organelles, cell membranes, secretory granules, and lysosomes. Caveole are specialized invaginations of the plasma membrane enriched with caveolin-1 (the nonmuscle isoform of a coat protein of caveole), Ca²⁺-ATPase, G proteins, and inositol trisphosphate receptors. Recently, enzymatic ecNOS activity was demonstrated in caveolar membranes. ecNOS colocalized with caveolin-1 in cultured microvascular cells, and antibodies to caveolin-1 immunoprecipitated ecNOS from the endothelium of myocardial capillaries. In addition to their role in endothytosis, the caveolin-rich membrane domains are thus also engaged in signal transduction.

Although ecNOS is a constitutive enzyme, its expression in vascular endothelium can be modulated by shear stress,
oxygen,24 transforming growth factor-β,25 cytokines,26 and various other factors (for a review see References 27 and 28). Regional differences in these modulating conditions might explain the wide variations in expression of ecNOS observed in endothelium of various cardiovascular segments. Heterogeneity of immunohistochemical ecNOS staining in endothelium has been reported in lung and renal vessels.25,26 In rat heart, NADPH-diaphorase staining was more intense in coronary arterial ECs than in venous ECs.6 It is not known whether ecNOS labeling of endocardial ECs and other cardiac ECs displays differences in its intensity or intracellular distribution.

In the present study, we have demonstrated, through whole-mount immunostaining and en face confocal microscopy, that ecNOS was present in all cardiac ECs. Staining was more intense in arterial and endocardial endothelium than in the endothelium of coronary veins and myocardial capillaries. This difference in staining was associated with heterogeneity in the size of the Golgi complexes.

Materials and Methods

For en face confocal laser scanning microscopy, rats were perfused with 2% paraformaldehyde in a HEPES buffer (mmol/L: NaCl 136.9, KCl 2.7, MgCl2·6H2O 0.5, KH2PO4 1.50, NaH2PO4·H2O 8.10, glucose 5.0, and HEPES 20, pH 7.2; total fixation time of 20 minutes; 5 minutes at 37°C and 15 minutes at room temperature). Strips of endocardium (right ventricle: septum and tendon end of papillary muscles, septum, outflow tract, and pulmonary valve; left ventricle: right and left ventricular free wall), and coronary vessels were isolated and prepared for immunocytochemistry. The periphery of the endocardial EC was outlined by an area, with a ringlike or more complex shape, of intense labeling corresponding to the Golgi complex was located near the stained nuclei. In each endocardial EC, a brightly stained spot displayed differences in its intensity or intracellular distribution. Usually, a Golgi complex consisted of an area, with a ringlike or more complex shape, of intense labeling and an unstained or lightly stained central dot. Double staining with ecNOS and Golgi 58k protein, a Golgi marker, confirmed that ecNOS had indeed labeled the Golgi complex. The periphery of the endocardial EC was outlined by an ecNOS-stained bandlike structure coinciding with PECAM labeling in double-stained preparations (Fig 2A and 2B). PECAM is known to label the whole depth of endothelial intercellular clefts. In the heart, all PECAM-labeled ECs expressed ecNOS. The three primary ecNOS antibodies used in the present study yielded similar results. Staining with the polyclonal antibody from Transduction Laboratories had a more granular appearance. Immunofluorescence staining of endocardial ECs revealed cytoplasmic labeling that outlined the unstained nuclei. In each endocardial EC, a brightly stained spot corresponding to the Golgi complex was located near the nucleus (Fig 1A and 1B). Occasionally, one cell contained two Golgi complexes. Usually, a Golgi complex consisted of an area, with a ringlike or more complex shape, of intense labeling and an unstained or lightly stained central dot. Double staining with ecNOS and Golgi 58k protein, a Golgi marker, confirmed that ecNOS had indeed labeled the Golgi complex. The periphery of the endocardial EC was outlined by an ecNOS-stained bandlike structure coinciding with PECAM labeling in double-stained preparations (Fig 2A and 2B). PECAM is known to label the whole depth of endothelial intercellular clefts. In the heart, all PECAM-labeled ECs expressed ecNOS. The width of the PECAM bands (hence, of the intercellular clefts) was larger in endocardial ECs than in arterial and venous ECs, thus confirming previous ultrastructural observations.22 In several endocardial ECs, PECAM labeling was also observed in Golgi complexes.

Immunostaining for caveolin-1, with monoclonal or polyclonal antibodies, showed that peripheral borders of endocardial ECs were nearly completely devoid of caveolin labeling (Fig 3A, 3B, and 3C). The pattern of these dark unstained peripheral areas was similar to PECAM-1 labeling. Many
endocardial ECs also contained juxtanuclear caveolin labeling. Subadjacent cardiomyocytes were not labeled (Fig 3C). The juxtanuclear labeling was more evident after staining with the monoclonal anti–caveolin-1 antibody.

Although nNOS labeling was not detected in endocardial ECs, it was prominent in neurons and nerve fibers of ganglia in the right atrium near the superior vena cava (not shown).

PECAM and ecNOS labeling revealed considerable differences in endocardial EC size between various areas of the right and left ventricular endocardial endothelium. In some hearts, larger endocardial ECs, which were usually observed in the right ventricular outflow tract and septum, possessed a larger Golgi complex than the small endocardial ECs, which can be found on the tendon end of right ventricular papillary muscles or on left ventricular papillary muscles. In other hearts, however, the size of the Golgi complexes and the degree of cytoplasmic staining did not differ between various areas within and between the right and left ventricles.

Endothelium of Coronary Arteries

Optical sections through coronary vascular endothelium (Fig 1C and 1D) revealed a considerably different pattern of ecNOS staining than did sections through endocardial ECs. In low-power images, there was an absence of the staining of peripheral cell borders in arterial endothelium. Double staining with
ecNOS and Golgi 58k protein confirmed that the most brightly labeled structures in arterial ECs (Fig 2C and 2D) coincided with Golgi complexes. The Golgi complexes were usually located alongside the nucleus. As in endocardial ECs, a dark unstained area was present in the Golgi complexes of arterial ECs. Golgi complexes had a more elongated and frequently more complex shape in arterial endothelium than in endocardial ECs. In several arterial ECs, the Golgi complex consisted of nearly disconnected granule-like spots. In the endothelium of arterioles (Fig 1E), Golgi complexes had a shape and size similar to those in the endothelium of coronary arteries. On the other hand, ecNOS labeling of endothelium of the thoracic aorta had a pattern similar to that in endocardial ECs. Golgi complexes were somewhat smaller in aortic ECs (6.45±0.25 μm², mean±SE, n=53) than in coronary arterial ECs (8.40±0.23 μm², n=77; both mean±SE values from aorta and coronary artery were from the same rat).

Double-labeled preparations of coronary arteries and aorta demonstrated that each of the PECAM-labeled cells was ecNOS positive. In addition, double labelings of arterial ECs showed that peripheral PECAM staining usually did not coincide with ecNOS staining (Fig 4C). More intense staining of cytoplasmic and membranous structures of arterial ECs resulted from different levels of focusing. Areas where both stains colocalize should have a yellow color. Bar=10 μm. D and E, Confocal images from ecNOS-stained endocardial ECs (D) and coronary arterial ECs (E). Figure 4. A and B, Double immunolabeling of RECA and ecNOS of a cryostat section through the left ventricular wall. All microvessels labeled by RECA were ecNOS positive. C, En face optical section through arterial EC (arterial endothelium [AE]) after double labeling for PECAM-1 (green color) and ecNOS (red color). The green color of the peripheral bands shows the absence of colocalization with ecNOS. Areas where both stains colocalize should have a yellow color. Bar=10 μm. D through G, Confocal images from ecNOS-stained endocardial ECs (D and E), coronary arterial ECs (F), and venous endothelium (VE) (G) obtained by the same contrast and pinhole settings of the confocal microscope. In panel D, pixel-gray values were color-coded showing distinct Golgi complexes and peripheral bands. Other areas of the endocardial ECs (endocardial endothelium [EE]) were weakly labeled. Note the differences in intensity of labeling between varous endocardial ECs, which mainly resulted from different levels of focusing. In panels E and F, the pixel-gray values were color- and height-coded to appreciate better the differences in labeling intensity of the Golgi complexes in cardiac endothelium. High and yellow to white areas indicate zones of intense labeling. The peaks in arterial ECs (F), representing Golgi complexes, were larger and had pixel values higher than the peaks in venous ECs (G). In the cytoplasm between Golgi complexes, more green and light-blue pixel values were present in arterial endothelium than in endocardial ECs. Cytoplasmic labeling was weak in VE.
endocardial ECs, aortic ECs also showed ecNOS-positive peripheral borders.

Caveolin-1 labeling of arterial ECs consisted of a patchy distribution of intense or weakly stained and unstained cellular areas (Fig 3D). Nuclei were more difficult to observe in arterial ECs than in endocardial ECs. The peripheral borders of arterial ECs were caveolin negative, but the unstained bands were much thinner than in endocardial ECs and sometimes difficult to discern by their irregular appearance and by the presence of unstained cellular zones. nNOS labeling was not detected in the endothelium of coronary arteries.

**Endothelium of Myocardial Capillaries**

Optical sections through myocardial tissue strips showed rather weak cytoplasmic ecNOS labeling of capillary endothelium. The ecNOS labeling outlined the nuclei (Fig 1F). The labeled Golgi complexes occupied a small area and usually had a circular or oval shape. Peripheral borders of the capillary ECs were not discernible. Double-stained preparations showed complete overlap of ecNOS labeling and RECA labeling of the ECs in myocardial microvessels (Fig 2E and 2F). RECA is an antibody that labels endothelium of the entire vasculature in all organs and tissues in the rat, including the sinusoidal endothelium and the high endothelium in lymphoid vessels.33 Cryostat sections of myocardial tissue also showed complete overlap of ecNOS and RECA labeling of ECs in myocardial microvessels (Fig 4A and 4B). No differences in the pattern of ecNOS labeling of microvascular endothelium were observed between right and left ventricular myocardium. No specific staining was detected in cardiac myocytes. The degree of staining in cardiac myocytes after ecNOS labeling was similar to that in negative controls, where primary antibodies were omitted. In myocardial tissue strips, the polyclonal ecNOS antibody from Transduction Laboratories produced a higher aspecific background in cardiac myocytes and interstitial tissue than the other primary antibodies.

After PECAM-1 staining, capillaries were rather weakly labeled. In some capillaries, thin bands could be observed, suggesting the existence of thin peripheral borders. In contrast to PECAM-1 staining, immunostaining for caveolin-1 produced very intense labeling of myocardial capillaries (Fig 3E). Endothelium of myocardial capillaries was much more strongly stained for caveolin-1 than was endocardial or arterial endothelium. Adjacent cardiomyocytes were unstained.

**Endothelium of Coronary Veins**

Venous ECs were less intensely stained with ecNOS than were endocardial ECs and ECs of coronary arteries. A substantial increase of contrast and gain of the confocal microscope was necessary to visualize ecNOS staining in coronary veins. ecNOS-stained peripheral borders outlined venous ECs, displaying their typical elongated shape (Fig 1G). The cell borders were thin and weakly labeled; frequently, only one or both extremities of the cells were stained as intensely as the Golgi complexes (Fig 1H). Golgi complexes appeared small and had an unstained center. Golgi complexes were more frequently located underneath the nucleus in venous ECs than in endocardial ECs and arterial ECs. Caveolin-1 staining yielded similar images in venous ECs and arterial ECs. The unstained peripheral bands were more distinct in venous ECs than in arterial ECs (Fig 3F).

**Image and Statistical Analysis**

The intensity of cytoplasmic ecNOS staining in cardiac ECs was compared by using three-dimensional color-coded plots of pixel-gray values from images taken with the same contrast and gain settings and width of the confocal aperture on the Bio-Rad MRC-600 (Fig 4E through 4G). Golgi complexes in arterial ECs were not only larger but also more intensely labeled than Golgi complexes in endocardial ECs and especially in venous ECs. Between the Golgi complexes, more green-coded and light-blue–coded pixels were present in arterial ECs than in endocardial ECs. Venous ECs contained the least cytoplasmic labeling around the Golgi complexes.

The area of the Golgi complexes, detected by ecNOS staining, was nonuniformly distributed in cardiac ECs. The distributions of the area of Golgi complexes in endocardial (right ventricle and septum), arterial, and venous cells (n=5 rats, 701 measured areas) were significantly right-skewed. Logarithmic transformation was used to normalize these distributions; the variances were stabilized by the same procedure. Coefficients of variation for the endothelial Golgi complex area, derived from the within-rat variation in a one-way ANOVA on the transformed data, were similar (Levene test): 25.8%, 26.8%, and 26.8% for endocardial, arterial, and venous ECs, respectively. The means of backtransformed Golgi complex area were significantly different among endocardial, arterial, and venous ECs (Fig 5A). Measurements of Golgi complexes in the endothelium of myocardial capillaries yielded similar or smaller values than found in venous ECs.

The size of the Golgi complexes was not correlated with the cellular size. PECAM and ecNOS labeling revealed a considerable variation in endocardial EC size between various areas of the right and left ventricular endocardium. Since cellular borders could not be detected in arterial ECs after ecNOS staining, the reciprocal of cell density was used to estimate the area of ECs. Cellular area was analyzed in a manner similar to that used for the Golgi complex area. The coefficients of variation were 16.1%, 10.5%, and 16.8%, and cellular area was estimated to be $666 \pm 40$ (mean $\pm$ SE), $350 \pm 28$, and $439 \pm 7$ $\mu m^2$ for endocardial, arterial, and venous ECs, respectively. These estimated values were of the same magnitude as measurements of the cellular area from rat endothelium, where cellular borders were visualized after staining for actin or PECAM (References 32 and 34, and authors’ unpublished data, 1996). Fig 5C confirms the lack of correlation between the Golgi complex area and the cellular area in rat cardiac endothelium. The largest Golgi complexes were present in arterial ECs, which had the smallest cellular area.

The shape of the Golgi complexes was nonuniformly distributed in cardiac endothelium. For the maximal cord of Golgi complexes, the coefficient of variation in arterial ECs, 31.8%, was significantly larger than that for endocardial or venous ECs, 23.7% and 26.9%, respectively. The maximal cord in endocardial ECs was significantly different from that in arterial or venous ECs (Fig 5B).
Our immunofluorescence data confirmed and extended previous observations of histochemical NOS detection in the heart. When NOS was determined by the histochemical NADPH-diaphorase method, it was demonstrated that coronary arteries in pig hearts manifested more NOS activity than did coronary veins. Weaker ecNOS immunoreactivity and nNOS in endocardial ECs and in the endothelium of coronary arteries of rat hearts. Further investigations are needed to support the absence or presence of ecNOS in endocardial ECs or in the endothelium of coronary arteries.

Immunofluorescent staining of ecNOS strikingly labeled the Golgi complexes of ECs, as validated by double immunostaining for ecNOS and Golgi 58k protein. The subcellular distribution of ecNOS in Golgi complexes has also been documented in cultured endothelium using NADPH-diaphorase staining. Spots of the histochemical reaction product have also been shown to be visible in situ, near nuclei in sections of endocardial endothelium (see Fig 2B of Reference 8). Further biochemical analysis and oligonucleotide-directed mutagenesis determined that cotranslational N-myristoylation of ecNOS is necessary for ecNOS Golgi targeting and that posttranslational palmitoylation influences ecNOS targeting into caveolae. In cardiac endothelium, the largest Golgi complexes were present in arterial and endocardial ECs. The size of the Golgi complexes was not related to the surface area of the ECs. Large and flattened ECs might possess a flattened and hence apparently larger Golgi complex. However, the largest Golgi complexes were present in the arterial ECs that had the smallest surface area. The size of the Golgi complex is probably a marker of the synthetic activity of a cell. Coronary arterial ECs and endocardial ECs in the rat might have a higher synthetic activity than do capillary and venous ECs.

Previously, ultrastructural studies in teleosts demonstrated that endocardial ECs contained more ribosomes, endoplasmic reticulum, and larger Golgi complexes than did ECs from myocardial capillaries, suggesting that endocardial ECs are more involved in protein synthesis than are capillary ECs. Ultrastructural investigation of endocardial ECs in rats also demonstrated well-developed Golgi complexes with many juxtanuclear coated and uncoated vesicles. In ECs, the Golgi complex is involved in the synthesis of various proteins, ranging from extracellular matrix components like collagen to more typical endothelial components like the intercellular adhesion molecule PECAM-1 (Fig 2A), the von Willebrand factor, and coagulation factor S. Hypertrophied Golgi complexes and proliferation of endoplasmic reticulum in endothelium are characteristic for embryological processes, for endothelial regeneration, and in dysfunctional endothelium during various pathological conditions, such as hypercholesterolemia, endotoxin injury, chronic ethanol administration, and hydrostatic edema formation. The size of the arterial endothelium have also been described for lung and kidney. Moreover, stronger NADPH-diaphorase staining was present in the endothelium of efferent arterioles than in the endothelium of afferent arterioles of mammalian kidneys. The strong NADPH-staining in ECs of efferent arterioles was ascribed to the mixed presence of ecNOS and nNOS (NOS type I). The presence of the neuronal isoform of NOS has also been reported in endothelium from rabbit aorta and from rat coronary and pulmonary arteries. However, since macrophage-like cells were also labeled, the authors could not exclude the possibility that the nNOS antibody also reacted with other NOS isoforms. Our present results with a commercial anti-nNOS polyclonal antibody did not provide evidence of nNOS expression in endocardial ECs and in the endothelium of coronary arteries of rat hearts.
Golgi complex can thus be used as a marker for the functional status of ECs.

Intense labeling and the large size of Golgi complexes in endocardial ECs and in coronary arterial endothelium indicated a high rate of eNOS production and could be related to the strong cytoplasmic eNOS expression in these cells. Experiments in cultured ECs have demonstrated that eNOS expression can be modulated by shear stress, transforming growth factor-β, protein kinase C, tumor necrosis factor-α, oxygen, and the proliferative state. In the heart, differences in shear stress could explain the strong and weak eNOS expression of arterial and venous ECs, respectively. Experiments with various reporter systems and direct measurements of NO have demonstrated that laminar shear stress increases the endothelial release of NO.

Fluid shear stress increases not only ROS mRNA and protein but also endothelial superoxide dismutase, which further augments the local release of NO. Shear stress might thus be involved in the differential expression of eNOS in arterial, capillary, and venous ECs of rat hearts. What about endocardial ECs? By comparison, laminar fluid shear stress is probably not high along the surface of endocardial ECs; nevertheless, although less pronounced than arterial ECs, endocardial ECs also manifested strong eNOS expression. The endocardial surface might be more subjected to turbulent flow, yet this type of flow does not increase NO mRNA and NO release in cultured human umbilical vein ECs. In endocardial ECs, mechanical strain by three-dimensional changes of the inner wall during the cardiac cycle might influence eNOS expression. ECs cultured on flexible substrates and subjected to cyclic strain showed an increase of NO mRNA, protein, and NO production.

However, we did not observe significant differences in eNOS expression between various areas of the endocardial endothelium known to undergo distinct differences in mechanical deformation during the cardiac cycle, eg, the tendon end of right ventricular papillary muscles and the atrioventricular valves. Endocardial ECs covering these highly elastic structures are smaller and have a cytoskeletal organization different from that of other endocardial areas, but they did not show consistent differences in eNOS labeling and in the size of Golgi complexes. Remarkably, freshly isolated ECs from large porcine coronary arteries do express more eNOS protein and produce more NO than do ECs from resistance arterioles, although both are subjected to similar shear stress. Factors other than shear stress might thus influence the expression of eNOS in cardiac endothelium.

A distinct finding in endocardial ECs and in coronary venous ECs, but much less in coronary arterial endothelium and not in capillary endothelium, was the presence of bands of eNOS labeling along the cell periphery. These bands coincided with the area of intercellular contacts, as demonstrated by double immunostaining with PECAM-1. PECAM-1 is an intercellular adhesion molecule that labels the whole depth of endothelial intercellular clefts. Remarkably, a similar labeling of peripheral borders in endocardial ECs was present after NADPH-diaphorase histochemical staining (see Fig 2B in Reference 8). This suggests enzymatic eNOS activity along the peripheral borders of endocardial ECs. Previous studies involving cultured ECs have demonstrated that an NO-induced increase of cGMP decreases paracellular permeability. NO production by the peripherally located eNOS in endocardial and venous ECs might thus be involved in the regulation of paracellular permeability.

Surprisingly, the eNOS-rich intercellular boundaries of endocardial ECs were not stained or were only weakly stained for caveolin. Caveolin-rich microdomains in the plasmalemma are sites where eNOS and various other molecules involved in transduction mechanisms are situated. Depending on the type of the vascular bed, the number of caveoleae can range from 10 to 460/μm² of EC surface. Besides an association with plasma membrane caveoleae, caveolin also resides in the Golgi complex and appears to cycle between these two compartments. Caveolin staining, in contrast to eNOS labeling, was much stronger in the endothelium of myocardial capillaries than in endocardial and arterial ECs. This apparently confirms that compared with large vessel endothelium, microvascular endothelium contains more caveoleae. Transmission electron microscopy revealed less caveolar vesicles and pits in endocardial ECs than in the endothelium of myocardial capillaries. Further work is clearly needed to determine the correlation between the presence of caveolin and eNOS in the various vascular beds. The distinct eNOS labeling and absence of caveolin labeling of peripheral borders in endocardial ECs suggest that eNOS might also be associated with other membrane components or with cytoskeletal components.

The distinct eNOS labeling of Golgi complex and peripheral borders in endocardial ECs raises the question of where the active pool of eNOS is located. Our results cannot address the existence of enzymatic eNOS activity in a particular compartment. The enzymatic activity of Golgi complexes is probably reflected by its NADPH-diaphorase activity and staining of ECs in vitro and in situ. Fractionating studies suggest the presence of mature eNOS in EC membranes and in intracellular membranes. Cytosolic fractions contain a substantially lower eNOS activity than does the particulate fraction. Brefeldin-induced disassembly of the Golgi complex in cultured ECs results in a loss of NADPH-diaphorase activity and in a significant decrease of NO production. This decrease was already significant at 15 minutes and was maximal at 90 minutes after incubation with brefeldin. This decrease is probably not an effect of a blockade of eNOS cycling from Golgi complex to the plasma membrane, since the half-life of eNOS protein measures 20 hours. The mechanisms of the brefeldin-induced inhibition of NO production are not known. The inhibition might result indirectly from interference with a cofactor needed for enzyme activity or from blocking of the active site. However, these data probably support the hypothesis that Golgi complexes, besides the caveolar membrane domains, are important sites of NO production.

In conclusion, the present study demonstrated considerable nonuniformity in the expression of eNOS and of the size of Golgi complexes in cardiac endothelium. The presence of intense eNOS-labeled and large-sized Golgi complexes in endocardial ECs and coronary arterial cells is in accordance with a more intense cytoplasmic eNOS labeling and is suggestive for a higher eNOS activity in these cells than in
coronary venous and myocardial capillary ECs. The lack of caveolin labeling and the presence of ecNOS labeling along the periphery of endocardial and venous ECs suggest that ecNOS might be associated with other membrane components or with parts of the cytoskeleton. Further investigations in disease states or during embryological development might allow a better understanding of ecNOS distribution and the size of Golgi complexes in cardiac endothelium.

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References


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