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Coronary ischemia-reperfusion: role of nitric oxide and endothelin-1. A Review

Title in Spanish: *Ischemia-reperfusión coronaria: papel del óxido nitro y endothelina-1. Una Revisión*

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ABSTRACT: This Review focus on the myocardial ischemia-reperfusion, paying particular attention to the role of nitric oxide (NO) and endothelin-1 (ET-1) in the regulation of the coronary circulation under normal conditions and after ischemia-reperfusion. Coronary atheromatosis is usually induced by endothelium dysfunction/damage, and is the main cause of acute coronary syndromes (e. g., acute myocardial infarction, AMI). The assessment of endothelial function of patients with coronary artery disease may provide useful information. The most effective treatment of AMI is timely reperfusion for restoring the blood flow to the ischemic myocardial territory, but this procedure may also damage myocardium (reperfusion injury), of which the pathophysiology and treatment remain uncertain. The interaction between NO and ET-1 may be relevant for regulating the coronary circulation, with predominance of NO over ET-1 under normal conditions. The coronary circulation plays a crucial role in ischemia-reperfusion since it is the cause and victim of consequences of this condition. Ischemia-reperfusion damages not only the myocardium but also coronary vasculature, including the endothelium, increases plasma levels of ET-1, induces functional predominace of ET-1 over NO, augments the coronary response to ET-1, and alters the role of endothelin receptors in this response. All these alterations may lead to dysregulation of coronary vasculature and the non-reflow phenomenon, which may underly reperfusion injury. Thus, ET-1 could be of significance in pathophsysiology of ischemia-reperfusion and reperfusion injury, and the use of antagonists for endothelin ETA/ETB receptors could protect the heart against reperfusion injury.

RESUMEN: Esta Revisión se centra en la isquemia-reperfusión del miocardio, prestando particular atención al papel del óxido nítrico (NO) y endothelina-1 (ET-1) en la regulación de la circulación coronaria en condiciones normales y tras la isquemia-reperfusión. La ateromatosis coronaria suele estar causada por disfunción/daño endotelial, y lidera las causas de los síndromes coronarios agudos (p. e., infarto agudo de miocardio, IAM). El tratamiento más eficaz del IAM es la reperfusión del territorio de miocardio isquémico, pero este procedimiento también causa lesión del miocardio (lesión por reperfusión), cuya fisiopatología y tratamiento siguen siendo inciertos. La interacción entre el NO y ET-1 es relevante en la regulación de la circulación coronaria, y en condiciones normales predomina el NO sobre la ET-1. La circulación coronaria desempeña un papel crucial en la isquemia-reperfusión puesto que ella es la causa y víctima de las consecuencias de esta situación. La isquemia-reperfusión daña no solo el miocardio, sino también el lecho vascular coronario, aumenta los niveles plasmáticos de ET-1, induce el predominio de la ET-1 sobre el NO, aumenta la respuesta coronaria alaET-1 y altera el papel de sus receptores en esta respuesta. Todo ello podría contribuir a la disfunción de la circulación coronaria y el fenómeno de no-reflujo y, como consecuencia, a la lesión por reperfusión. Así, la ET-1 podría ocupar un papel destacado en la fisiopatología de la lesión por reperfusión, y el uso de antagonistas de los receptores ETA/ETB podría proteger al corazón frente a la lesiones por reperfusión.

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1. INTRODUCTION

Ischemic heart disease is the most lethal of cardiovascular diseases, and it is one of the leading causes of morbidity and mortality in humans worldwide, including European Union and Spain. The social impact of this disease is considerable, not only because of the mortality that it causes, but also because of its consequent morbidity, loss of quality of life, and high economic cost. This impact is largely due to its pathophysiological mechanism, namely, cardiomyocyte death.

In ischemic heart disease, cardiomyocyte death almost always occurs as consequence of severe and prolonged myocardial ischemic events, which are mostly due to complications from atherosclerotic plaques in epicardial coronary arteries, causing an acute coronary syndrome. Coronary atherosclerosis develops in a clinically silent manner over years and only causes clinical symptoms when the arterial lumen is greatly narrowed, either due to atherosclerotic plaque growth or the development of intracoronary thrombosis induced by plaque complication, resulting in a loss of endothelial

continuity due to erosion, fissure, or endothelial rupture. Endothelial dysfunction and damage is a main factor for the formation of atheromatous plaques and development of coronary artery disease. Many clinical trials have suggested that lifestyle and pharmacologic interventions are effective in attenuating atherosclerotic disease progression and events development in coronary vasculature. Thus, preventing the appearance of atherosclerosis coronary plaques or their progression should be the first line of action against the disease.

The atheromatous plaques, by producing severe stenosis and/or thrombosis of a coronary artery, may induce a pronounced reduction (>80%) of coronary blood supply in the distal territory of myocardium supplied by the artery affected, thus causing acute myocardial infarction (AMI). This entity frequently occurs in persons >45 years old, of which ~1/3 die within 28 days after beginning clinical symptoms. Studies of sex differences in long-term mortality after AMI have reported mixed results. Main determinants in evolution of patients with AMI are individual factors (e. g., age of patient), size of infarct, and time elapses between symptoms felt by patient and instauration of an effective treatment. The treatment should be directed to salvage the maximum amount possible of myocardium, by applying the adequate treatment as early as possible. Ischemic myocardial injury may also occur in clinical conditions such as heart transplantation, cardiac bypass, and coronary stenting after acute myocardial infarction (for details, see References 1-5).

The incidence and fatality rates of an AMI are going down in developed countries as a result of better prevention and treatment. Basic and clinical researchers agree that the most effective treatment of AMI is timely (early) reperfusion by opening the stenosed/thrombosed coronary artery, thus restoring the blood flow to the ischemic myocardial territory. Timely restoration of blood flow to the ischemic zone of the myocardium limits infarct size and reduces mortality, and this reperfusion can be performed in patients, either by using thrombolytic agents (6) or percutaneous coronary angioplasty (7), and both of these procedures were initiated around 1975 (8). AMI is a dynamic process that frequently reaches the total extension of myocardium necrotized within 6h after initiating the artery occlusion. Myocardial reperfusion intents to stop expansion of myocardial damage, and this procedure represents a major step in treatment of patients with AMI. Reperfusion therapy is one of the most successful therapies of modern medicine. Today, primary percutaneous coronary angioplasty is the preferred strategy if the procedure can be performed by an experienced team within 90–120 min after the patient first presents (8). It is preferred because it can confer more rapid and complete revascularization, induces revascularization in a larger number of infarct-related coronary arteries and is associated with fewer bleeding complications. However, because access to invasive facilities is limited, thrombolytic therapy is still employed in many centers worldwide (8). In advanced countries, patients are now receiving the benefits of reperfusion therapy, and reducing time between symptoms onset and reperfusion therapy, is at present one of the main goals in the management of patients with AMI (9).

Also, basic and clinical researchers agree that not all it is favorable with myocardial reperfusion, and as Braunwald and Kloner written at 1985, myocardial reperfusion is “a double edge sword” (10). Whereas it is very difficult to study the pathogenesis of human coronary syndromes such as AMI, experimental models have provided good reproducible means to explore different aspects of the events that occur after myocardial ischemia-reperfusion. Around the 1960s, R. B. Jennings and cols. brightly demonstrated in laboratory animals the consequences of a temporary occlusion of a coronary artery, as well as those of reperfusion by releasing this coronary occlusion (11, 12). They observed that reperfusion may also damage myocardium exposed to ischemia, and this damage is added to myocardial damage caused by ischemia itself, and it known as reperfusion injury (13). This phenomenon has been also described in the clinical setting (10).

The coronary circulation plays a crucial role in the development of the effects of ischemia-reperfusion on the heart tissue. Indeed, the coronary circulation is the cause of the consequences of interruption of blood supply to cardiac tissue, as coronary artery disease is culprit of this interruption, and also it is victim of these consequences of blood flow deprivation as ischemia-reperfusion damages not only cardiomyocytes but also coronary vasculature. Furthermore, the coronary circulation is also protagonist in the process of reperfusion aimed to reduce the consequences of ischemia-reperfusion. Therefore, the coronary circulation should be considered with attention as it could play a central role in path physiology of myocardial ischemia-reperfusion and reperfusion injury, and also should be considered as a target of therapeutical strategies for cardioprotection (14). However, spite of relevance of the coronary circulation in ischemia-reperfusion and reperfusion injury, this vasculature has received little attention in literature about this issue (14). The present Review focus on myocardial ischemia-reperfusion, paying particular attention to the coronary circulation and to role of nitric oxide and endotheli-1 in the regulation of this vascular bed under normal conditions and after ischemia-reperfusion. Also, it will be considered therapeutical strategies for ischemia-reperfusion injury, with greater mention to antagonists for endothelin receptors.

2. THE CORONARY CIRCULATION

2.1. General considerations

Over the past several decades, the major factors determining myocardial perfusion have been elucidated, and this knowledge has been incorporated in the

management of patients with ischemic heart disease. The basic understanding of the flow mechanics of coronary stenoses have been also translated to the cardiac catherization laboratory where measurements of coronary pressure distal to a stenosis and coronary blood flow are routinely obtained (15, 16).

The coronary circulation provides oxygen and nutrients to heart tissue, and the myocardium is very sensitive to oxygen deprivation. Coronary blood flow is determined, essentially, by aortic pressure, myocardial metabolism, neural factors, circulating vasoactive substances, and endothelial factors (1, 17, 18). Myocardial function is closely coupled to coronary blood flow and oxygen delivery, thus balance between oxygen supply and myocardial demand is a critical determinant of the normal heart function (1, 17-19). Figure 1shows a schematic representation of different aspects of the human coronary circulation.

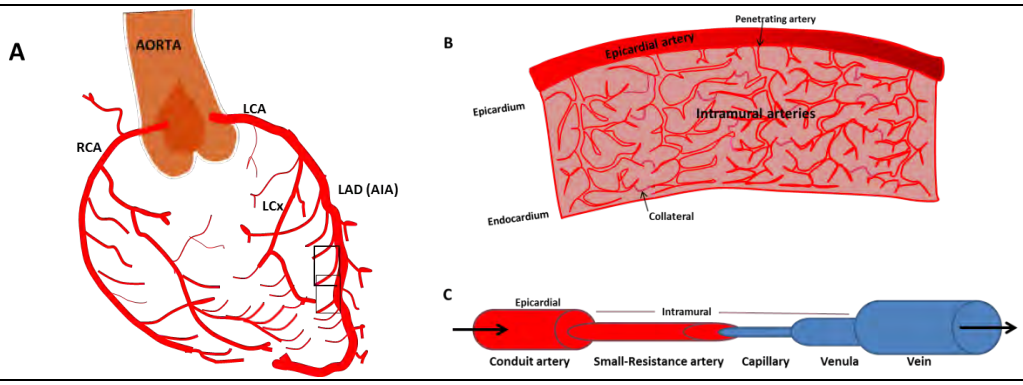

Figure 1. Schematic representation of the human coronary circulation: A) Drawing of the coronary circulation; LCA=left coronary artery; RCA=right coronary artery; LAD (AIA) =left anterior descending coronary artery; LCx=left circumflex coronary artery. B) Drawing of epicardial and intramural arteries; in the normal hearts, each area of myocardium is usually supplied by a single coronary artery and it does not have functional collaterals; and C) Drawing showing the different segments of coronary vascular system.

In the human heart, the blood supply to the myocardium is provided by the two main coronary arteries that arise from the aorta: the left coronary artery and the right coronary artery; the left coronary artery branches to the left anterior descending and circumflex coronary arteries (Figure 1A). The left and the right coronary arteries are epicardial arteries (1-3 mm in diameter), they divide on the surface of the heart in a base to apex direction, and they are considered conduit arteries. These arteries send tributaries (400-1, 500 µm in diameter) which penetrate transmurally through the myocardial wall from the outer epicardium to the inner subendocardium (Figure 1B). After penetrating the myocardium, they branch to small arteries and arterioles (75-200 µm in external diameter) which are the primary sights of coronary vascular resistance. From the arterioles, a dense network of capillaries arises, running parallel with the cardiomyocytes (~ 3, 500 capillaries/mm^2), with a gradient of vascularity favoring the endocardium; ratio of capillaries to cardiomyocytes is ~1:1. In addition, coronary mycrocirculation includes capillaries and postcapillary venules (Figure 1C). The coronary venous system is largely a mirror image of the coronary arterial system.

Under normal conditions, there is not measurable pressure drop in the epicardial arteries, indicating negligible conduit resistance. When a significant epicardial artery narrowing is present (>50% diameter reduction), the fixed conduit artery resistance begins to contribute significantly to total coronary vascular resistance and, when these arteries are severely narrowed (>80% diameter reduction), it may reduce resting blood flow. The major component of coronary vascular resistance under normal conditions mainly arises from small arteries and arterioles, consequently called resistance vessels. This resistance is dynamic and distributed throughout the myocardium across a broad range of vessel sizes (50-400 µm in diameter). Because the diameter of these vessels, and hence their resistance can be changed by passive and active mechanisms, they play a pivotal role in the regulation of coronary blood flow. There is normally little resistance contributed by capillaries and coronary venules, and their resistance remains fairly constant during changes in vasomotor tone (16-18).

Under resting conditions, a vasomotor tone exists to match oxygen delivery to metabolic need, and when elevated metabolic demand occurs, coronary blood flow can increase 5-fold primarily through arteriolar vasodilatation. This vasodilator reserve can also be utilized to maintain blood flow when perfusion pressure changes (16-18). When a 50% stenosis is present in a coronary conduit artery, this stenosis has little effect on blood flow due to autoregulatory reductions in arteriolar resistance (1, 16-18). However, during exercise or higher stenosis (~80%), blood flow may be compromised when arteriolar dilation is exhausted. Thus, conduit and resistance vessels work in harmony to defend myocardial perfusion. The inability of the microcirculation to compensate for changes in metabolism and perfusion pressure is one reason that examination of coronary arteriolar function is so important. There are a series of

collateral blood vessels that interconnects arteries, and under normal conditions these blood vessels have not functional connexions, but under some pathological conditions (e. g., arterial stenosis-obstruction) may provide functional salvage pathways of potentially ischemic areas of the myocardium (1, 16-18).

After ischemia-reperfusion, the coronary circulation deserves attention because of it is damaged in different degree and it is a prime determinant of myocardial ischemia-reperfusion injury: it determines the area at risk, the duration of myocardial ischemia, the residual blood flow through collaterals, the microvascular obstruction and the restoration of coronary blood flow during reperfusion. Also, the coronary circulation is the site of cardioprotective interventions with intermittent coronary occlusion/reperfusion. Inadequate consideration for the coronary circulation might easily diminish the magnitude of differences in infarct size between the possibly protected group and the control group (14).

2.2. The endothelium

The artery wall has three layers, which are termed, from outer to inside, adventitia, media and intima. The intima is covered by the endothelium, which is a single layer of cells lining the inside of vascular wall. Before 1980, the endothelium was merely considered as an anticoagulant sheet of cellophane, and at present it is accepted that the endothelium plays a crucial role in homeostasis of cardiovascular system. The healthy endothelium is able to produce a range of substances that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation (19, 20).

In 2009 P. M. Vanhoutte wrote: “First, there was Robert Furchgott. He simply and brilliantly demonstrated that endothelial cells play a pivotal role in relaxations evoked by acetylcholine in isolated arteries, and do so by activating muscarinic receptors of these cells” (21). In the late 1970s and early 1980s two cornerstone observations were made: the first, by Furchgott and Zawadzki, was the discovery of the "obligatory role of the endothelium in vasorelaxation by acetylcholine" (22), and the second, by Hickey and col., was the first description and characterization of a vasoconstrictor polypeptide produced by endothelial cells in culture (23). From the seminal observation by Furchgott and Zawadzki in 1980 (22), an exponential number of studies have been performed by many researchers, in many laboratories through worldwide, and using different vascular beds and different experimental preparations (isolated blood vessels, isolated organs, anesthetized and unanesthetized animals). The intense laboratory and clinical research have let us to know that the endothelium can produce and release vasodilator and vasoconstrictor substances, as well as to know some pathways that regulate endothelial-vascular smooth muscle communication (19). The reading of an interesting and nice article about the history of this issue, related by R. F. Furchgott, is recommended (24).

The endothelium constitutes a large organ, and it is formed by approximately 10^{13} cells, which represents 1, 5% of the total body mass (25). The endothelium derives from mesoderm, and in most of vascular beds it forms a continuous and non fenestrated layer that covers the internal surface of the blood vessel wall. When it is damaged, for instance after atherosclerosis, can be replaced by regenerated endothelial cells that may not have the same properties as normal cells (26, 27). The endothelium can produce and release endothelium-derived relaxing factors such as nitric oxide (NO), prostacyclin (PGI2), endothelial derived hyperpolarizing factor (EDHF), hydrogen peroxide, carbon monoxide, hydrogen sulphide, C-t natriuretic peptide, and others; of these factors the most relevant seem to be NO, PGI2 and EDHF. The endothelium also can produce and release endothelium-derived contracting factors such as endothelin peptides, angiotensin II, thromboxane A2; in some circumstances hydrogen peroxide and prostacyclin can produce vasodilatation or vasoconstriction (19, 25). Under normal conditions predominates the release of vasodilator substances, particularly NO and, in consequence, the normal endothelium is pro-vasodilator. However, under pathological conditions in which the endothelium is damaged (i. e., atherosclerosis) secretion of vasodilator substances are reduced, whereas the release of vasoconstrictor substances such as endothelin-1 (ET-1) may be augmented, the endothelium being now pro-vasconstrictor (19, 25, 26) (Figure 2).

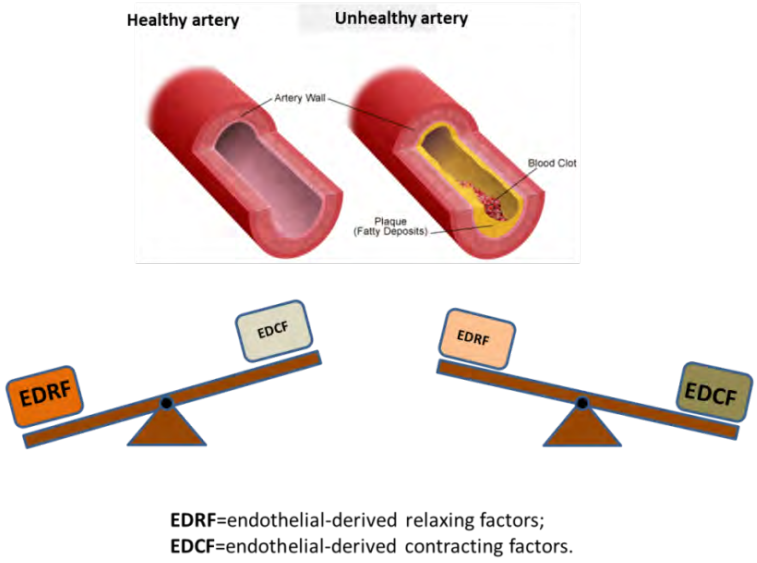

Figure 2. Schematic representation of the relation of the endothelial release of relaxing and contracting factors in healthy, normal arteries (left), and unhealthy, diseased arteries (right).

Early changes in endothelial function are indicators of future cardiovascular morbidity and mortality. Endothelial cell dysfunction related with aging in healthy persons is linked to telomere shortening, but it could be accelerated by risk factors, such as atherosclerosis, arterial hypertension, smoking, diabetes mellitus, obesity, and mechanical stress of a high heart rate (26-30). Such insults accelerate a proatherogenic phenotype (30-32). The statement "You are only as old as your endothelium", attributed to Dr. Rudolf Altschul (1901-1963), may be right.

Our understanding of the role of the endothelium in the coronary vasculature under normal and pathological conditions is very notable (1, 15-18, 20, 30, 31). Due to its hemodynamic characteristics, the coronary vasculature may be the prime site for endothelial dysfunction (18) and displays an unusual gene pattern compared with other blood vessels, with lower endothelial NO synthase (eNOS) and higher of ET-1 mRNA expression (32). This pattern predisposes coronary vasculature to endothelial dysfunction and atherosclerosis, and coronary arteries are a main target for the aging and effects of cardiovascular risk factors. Summarizing, the coronary vasculature is prone to predominate the effects of ET-1 over those of NO with aging, cardiovascular risk factors and some pathological situations.

The first demonstration of endothelial dysfunction in atherosclerotic coronary arteries using intracoronary infusion of acetylcholine (Ach) and quantitative coronary angiography dates back to 1986 (33). This observation drew attention to the functional manifestations of atherosclerosis such as exaggerated vasoconstriction as a consequence of poorly functioning endothelium. Later, less invasive techniques were developed using mainly the forearm circulation as a surrogate for coronary arteries (30, 31, 34, 35).

Atherosclerosis, the main cause of acute coronary syndrome, courses with endothelial dysfunction, and this dysfunction could play a relevant role in pathophysiology of myocardial ischemia-reperfusion. And also, myocardial ischemia-reperfusion by itself is a clinical and laboratory entity that may be accompanied by damage and dysfunction of coronary vasculature, particularly of the endothelium (see below). These ideas support the interest for investigate the functional aspects of the coronary circulation after myocardial ischemia-reperfusion in order to know the pathophysiology of this condition, as well as to design therapeutic strategies to ameliorate the consequences of acute myocardial infarction and reperfusion injury, and thus the outcome of patients with these abnormalities.

In the past decade, many studies have suggested that the noninvasive assessment of endothelial function may provide useful information for individual patient risk, progress, and guidance of therapy.

2.3. Nitric oxide (NO)

In mammals, including humans, nitric oxide (nitrogen oxide or nitrogen monoxide) is an important cellular signaling molecule involved in many physiological and pathological processes (for revision of this issue, see reference 36).

NO is a gas that has not colour, odour, or taste, with low solubility in water at room temperature and at atmospheric pressure. In blood, it reacts predominantly with molecules that have orbitals with unpaired electrons, which are typically other free radicals (superoxide ion, hydroxyl ion) or transition metals like heme iron (hemoglobin, myoglobin, cytochromes). Reactions of NO differ between in vitro and in vivo systems. In in vitro systems, the main degradation product of NO is NO^{2}- (nitrite), while in vivo the main product is NO^{3}- (nitrate)

as a consequence of the reaction of NO with hemoglobin.

This molecule can become highly toxic to tissues during some pathological conditions (e. g., ischemia and reperfusion). NO by reacting with anion superoxide (O2•−) forms peroxynitrite (ONOO−), which is a powerful oxidant and seems to be a determinant in the contrasting roles of NO in physiology and pathology. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms, which may commit cells to necrosis or apoptosis. In vivo, peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes mellitus, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders.

The regulation and synthesis of NO by mammalian cells has been the focus of many reviews (36, 37), as have been many of its physiological and pathological actions (38, 39). For a century, nitrovasodilators had been used clinically without understanding their mechanism of action. Alfred Nobel lamented the irony that he was taking nitroglycerin to treat angina after making his fortune developing dynamite.

As several studies suggested that the characteristics of NO and EDRF are similar, R. Furchgott proposed that the EDRF produced by endothelium is NO (40, 41), and Ignarro provided additional evidence supporting the identification (42, 43). Moncada et al. were able to directly measure NO produced in vivo (44).

The synthesis of NO from arginine is catalyzed by three forms of NO synthase (endothelial NO (eNO) synthase, neuronal NO (nNO) synthase, and inducible NO (iNO) synthase). The brain proved to be a rich source of NO synthesis and allowed the first NO synthase to be cloned and purified (45, 46). It is called nNOS or NOS1 as it was the first synthase to be cloned. The NOS1 gene has the most complex genomic organization in humans with multiple splice variants being produced (47). The second NO synthase to be cloned was isolated from macrophages and is known as NOS2 or iNOS (inducible NOS) because it is readily induced in many tissues by proinflammatory cytokines. Because NOS2 can be strongly induced by proinflammatory stimuli, it induces often a high production of NO. The first source of NO identified was the endothelium, but eNO synthase was the last to be cloned and is known as eNOS or NOS3. NOS3 binds to plasma membranes and is typically associated with caveolin (48). It is strongly activated by the entry of calcium through membrane-bound receptors and is also regulated by phosphorylation (49). NOS3 is also found in neurons and other tissues in addition to endothelium (36). NO is also produced by myocardium, and it is involved in several aspects of physiological myocardial function (e. g., excitation-contraction coupling; myocardial relaxation; diastolic function; the Frank-Starling response; heart rate; β-adrenergic inotropic response; and myocardial energetics and substrate metabolism) (50, 51). Figure 3 summarizes the source and the beneficial effects of NO on blood vessels.

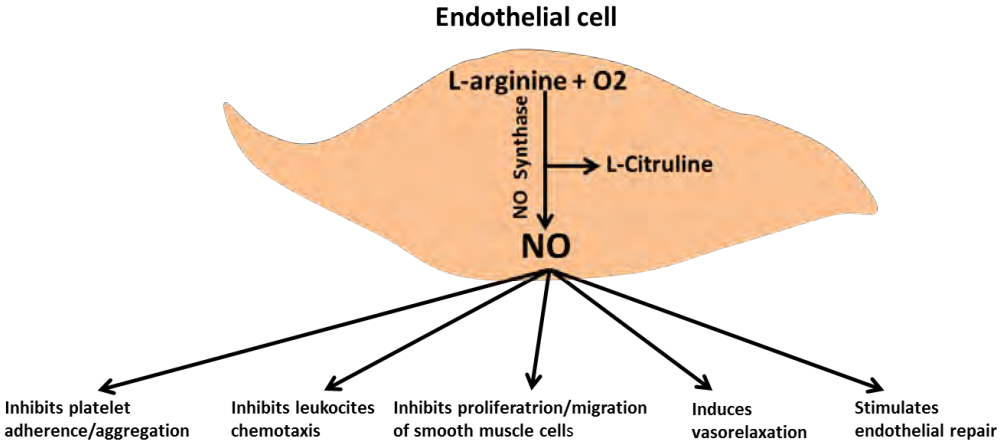

Figure 3. Summary of the source and beneficial effects of NO (nitric oxide) in blood vessels.

NO is an indicator of vascular endothelial function, and measurements performed in control (normal) humans provide that eNO synthase activity is 7-8 U/mL, and NO levels (expressed as NO^3) are approximately 60 µmol/L. NO is released from the endothelium under normal conditions as the endothelium acts as a sensor that is stimulated by flow changes, stretch, a variety of circulating vasoactive substances, and inflammatory mediators. The release of NO can increase or decrease in patients under certain pathological conditions. The release of NO can be pharmacologically inhibited by using analogues of arginine such as Nw-Nitro-L-arginine methyl ester (L-NAME) and L NG-monomethyl-L-arginine (L-NMA). These inhibitors have been used in laboratory experiments to explore contribution of NO in the regulation of vascular tone (52, 53).

In general, NO acts stimulating intracellular guanylate cyclase, and this enzyme converts GTP in cGMP. There are two types of guanylate cyclase: one is an integral membrane protein with an extracellular

domain and an intracellular catalytic domain that synthetizes cGMP; the other is cytoplasmatic (soluble guanylate ciclase) and is the isoform that is the form activated by NO. In the nervous system, NO acts directly to open cyclic nucleotid-gated channels, and it activates the cGMP-dependent protein kinase (36). NO, after it is released by the vascular endothelium, is free to diffuse into adjacent smooth muscle cells of the vascular wall, and in these cells directly activates soluble guanylate cyclase. This enzyme induces the formation of cGMP, which in turn activates cGMP-specific protein kinases that affect ion channels, calcium hemeostasis, or phosphatases, or all of these, causing relaxation of vascular smooth muscle (36).

As loss of NO bioavailability indicates a broadly dysfunctional phenotype across many properties of the endothelium, the assessment of its vasodilator properties resulting from NO and other molecules may provide information on the integrity and function of the endothelium. Interestingly, most, if not all, cardiovascular risk factors are associated with endothelial dysfunction and risk factor modification leads to improvement in vascular function (37, 54). Over the past 35 years, many methodological approaches have been developed to measure the (patho) physiological function of the endothelium in humans (55), but evaluation of endothelial function as a clinical tool in daily practice is not established yet.

From the beginning, endothelial function has been regarded as an important factor in the regulation of the coronary circulation and it may be of pathophysiological significance in several coronary diseases (1, 15-18). Endothelium-dependent vasodilatation has been found in coronary vessels from dogs (56) and man (57). Then it was reported that human coronary vessels in vitro release NO (58), that NO plays a significant role in modulating basal vasomotion and endothelial-dependent dilatation in the coronary circulation of dogs (59), and that the coronary vascular relaxation by NO, as well as by nitroprusside is associated with an increase in cGMP (60).

In our laboratory we have carried out a series of experiments to examine the functional role of NO in the coronary circulation under normal conditions, and they were performed using three laboratory models (anesthetized goats, isolated, perfused hearts from rats, and isolated coronary arteries from pigs). In these three models we found that in the coronary circulation, NO may be released under basal conditions, it produces a basal vasodilator tone, it mediates the vasodilation in response to Ach, and it reduces the response to vasoconstrictors such as ET-1. In anesthetized goats, L-NAME, injected by i. v. route, decreased resting coronary blood flow and increased systemic arterial pressure. These hemodynamic effects of L-NAME were partially reversed by L-arginine injected intravenously. We also found that during the effects of L-NAME, the coronary vasodilatation to Ach was attenuated, to sodium nitroprusside was increased, and to diazoxide was unaffected. Graded coronary hyperemic responses after 5, 10 or 20 s of coronary occlusion were increased during treatment with L-NAME. These results suggest: a) endogenous NO is involved in regulation of coronary circulation by producing a basal vasodilator tone; b) Ach-induced coronary vasodilatation is mediated, in part, by NO; and c) inhibition of basal endogenous NO production induces supersensitivity of coronary vessels to nitrovasodilators and enhances hyperemic responses after short periods of ischemia of the myocardium (61). This agrees with observations by others in the coronary circulation, and supports the idea that a vasodilator tone mediated by NO is present in the coronary circulation. The effects of sodium nitroprusside were increased during the action of L-NAME. Sodium nitroprusside has been used as a donor of exogenous NO and produces vasodilatation in a similar way to NO (62). Enhanced vasorelaxant responses to sodium nitroprusside and other nitrous compounds have been found in the absence of a functional endothelium or in the presence of L-arginine analogues, and this has been related to an increased sensitivity of guanylate cyclase in vascular musculature to exogenous NO when production of endogenous NO is reduced (63, 64). Therefore, it appears that the removal of endogenous NO in the vasculature could lead to increases in sensitivity to vasodilators that act by stimulating soluble guanylate cyclase. Reactive hyperemia after short ischemias of the myocardium is known to occur in the coronary circulation, and metabolic and hemodynamic factors that contribute to the hyperemic response have been explored. However, little is known about the role of the endothelium in the coronary hyperemic response (56, 59, 65).

Also in anesthetized goats, when L-NAME was intracoronarily injected, it reduced resting coronary blood flow without changing systemic arterial pressure. These effects of L-NAME were also partially reversed by L-arginine. Isoproterenol, adenosine and Ach, injected intracoronarily, increased coronary blood flow, and after treatment with L-NAME the increases in coronary blood flow induced by isoproterenol and Ach were reduced, whereas those induced by adenosine were increased further. Therefore, it is confirmed that in the coronary circulation NO produces a basal vasodilator tone under normal conditions, and it is suggested that NO may be an intermediate in the coronary vasodilatation in response to beta-adrenoceptor stimulation and Ach, and that the vasodilatation due to adenosine is potentiated during reduction of endogenous NO production (66) (See Figure 4).

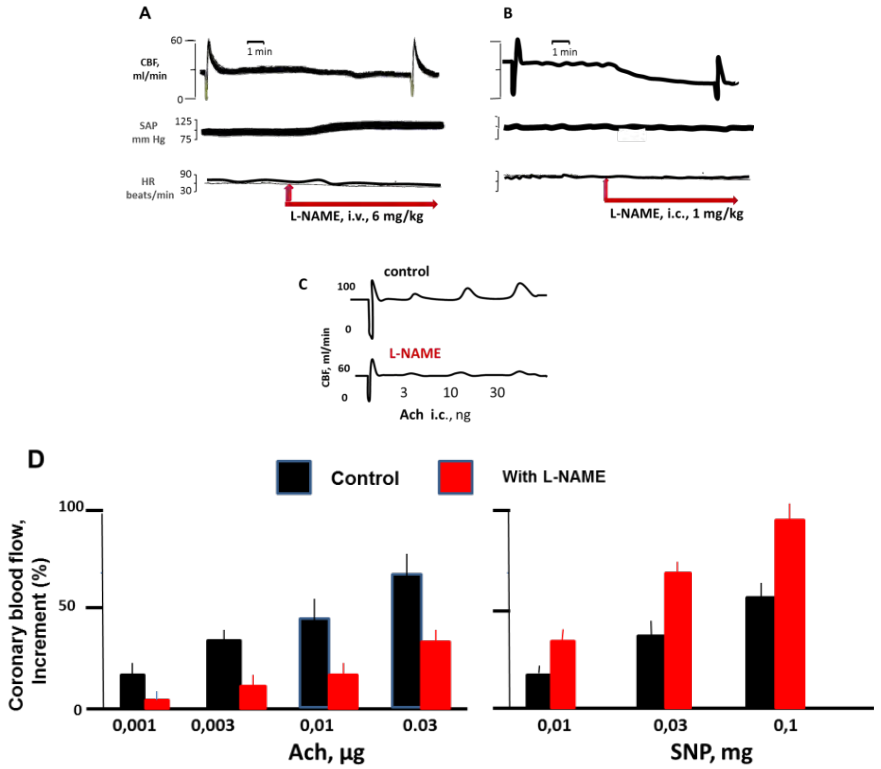

Figure 4. Actual recordings of coronary blood flow (CBF, electromagnetically measured), systemic arterial pressure (SAP) and heart rate (HR) obtained in anesthetized goats: A) during i. v. administration of the inhibitor of NO synthesis L-NAME; B) during intracoronary (i. c) administration of this substance; and C) showing the effects of intracoronary injections of acetylcholine (Ach) on coronary blood flow before (control) and after L-NAME injected by i. v. route; D) Summary of the effects of intracoronary injections of acetylcholine (Ach) and sodium nitroprusiate (SNP) on coronary blood flow in anesthetized goats under control conditions and after i. v. administration of L-NAME.

2.4. Endothelin-1 (ET-1)

Endothelin-1 is a member of the group of endothelins, which are a family of 21- amino-acid peptides, and they include endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3); they share structure homology with the snake venom sarafotoxin b and c (S6b and S6c) (67, 68).

The presence of an endothelium-derived constricting factor (EDCF) was perceived 1 year after the revelation of the endothelium-derived relaxant properties of the endothelium (69). However, it was 8 years later that Yanisagawa et al. identified an EDCF, ET-1 (70, 71). Two years later, two types of receptors for ET-1 were identified (72, 73), and shortly after the discovery of ETA y ETB receptors, M. Clozel et al. presented in 1993 the first orally active ET-1 receptor antagonist, Ro 46-2005 (74).

ET-1 is one of the most potent endogenous vasoconstrictor identified so far (67, 68). This peptide induces a long-lasting contraction of arteries of different regions, including coronary arteries, and it contributes to cardiovascular homeostasis through several pathways that impact on the regulation of basal vascular tone (75). It is released continuously, mostly from endothelial cells, by a constitutive and regulated pathway (76), and acts primarily as a paracrine regulator of vascular tone (75-78). In addition to endothelial cells, ET-1 is also produced by vascular smooth muscle cells, cardiomyocytes, leukocytes, macrophages, some types of neurons, and other types of cells (67, 77, 78). ET-1, at elevated concentrations, also induces inflammatory effects and promotes proliferation of vascular smooth muscle cells (67, 68, 79).

ET-1, ET-2 and ET-3 are encoded by different genes, on chromosomes 6, 1 and 2, respectively (67, 68). In endothelial cells, the product of the ET-1 gene is processed from a large precursor peptide of approximately 200 amino acid residues (preproET-1) to 39-amino-acid prohormone (big ET-1), which has about 1% of the activity of ET-1. This prohormone is then cleaved to form ET-1 by the action of two endothelin-converting enzymes (ECE-1 and ECE-2) (67, 68). Small amounts of big endothelin and ET-1 are secreted into circulating blood and into interstitial space of the blood vessel wall (67, 68). In normal human adults, plasma levels of ET-1 are relatively low (0. 7-6 pg/mL (75, 76). ET-1 is cleared from the circulating plasma, and the majority is retained by the lungs and cleared from the circulation by binding to endothelin ETB receptors (67, 68). ET-1 is not stored in secretory granules, and factors that active its secretion may be angiotensin II,

catecholamines, grow factors, hypoxia, insulin, lipoproteins, thrombin; contrarily, factors that inhibit its secretion are NO, atrial natriuretic peptide, prostaglandin E, PGI2 (67, 68). The concentration of ET-1 in plasma may increase in some cardiovascular diseases such as myocardial infarction, ischemia-reperfusion syndrome, and heart failure (78, 80).

The biological effects, including vascular effects of ET-1 may be mediated by two types of receptors, i. e., endothelin ETA and endothelin ETB; endothelin ETB receptors are subdivided in ETB1 and ETB2. ETA receptors are located in smooth muscle cells and coupled to protein G, and its activation leads to increment of cytoplasmic Ca, and then vasoconstriction (67, 68). The debate in literature exists whether or not ETA and ETB receptors should be blocked to provide most clinical benefit (67, 76, 81); this feature may be of clinical relevance, for example in treatment of myocardial reperfusion injury. The ETA receptor has been considered as the bad one, while the ETB receptor has been considered as the good one; this distinction is based on the role of ETB1 in the clearance of circulating ET-1 and on the finding that activation of this particular receptor produces vasodilation by releasing NO. Whereas the role of ETA receptors in vascular function is relatively well known, the role of ETB receptors is less known. Both ETB1 and ETB2 receptors are also a G-protein coupled receptor and have been identified in numerous types of blood vessels, including coronary arteries. ETB1 receptors, situated in the endothelium, mediate the release of relaxant factors (NO, PGI2, EDHF), and they may play a role in ET-1 clearance, and ETB2receptors, situated in vascular smooth muscle cells, mediate the increase of concentrations of intracellular Ca, protein kinase C, mitogen-activated protein kinase and other pathways of vascular smooth cells contraction and cell growth (67, 68).

In spite of many data demonstrating that ET-1 is a powerful vasoconstrictor, there are also data suggesting that under healthy conditions, ET-1 can also induce in vivo vasodilatation. In any case, the overall effects of ET-1 on vascular tone in vivo maybe the result of the balance between the effects mediated by ETA and ETB2 receptors located in smooth muscle cells, and the effects mediated by ETB1 receptors located in endothelial cells (82, 83), and this could dependent on whether blood vessels are healthy or unhealthy. Aging and pathological conditions (e. g., cardiovascular diseases) facilitate the predominance of the vasoconstrictor effects of ET-1.

Another cardiovascular function that may be of relevance is that ET-1 may be a regulator of cardiac physiology and pathology. Produced locally within the myocardium in response to diverse mechanical and neurohormonal stimuli, ET-1 seems to modulate cardiac contractility. During pathological cardiovascular conditions such as ischemia, left ventricular hypertrophy and heart failure, myocyte expression and activity of the ET-1 system is enhanced, allowing the peptide to both initiate and maintain maladaptive cellular responses. Both the myocardial acute and chronic effects of ET-1 are dependent on the activation of intracellular signaling pathways, regulated by the inositol-trisphosphate and diacylglycerol produced upon activation of the ETA receptor. Subsequent stimulation of protein kinases C and D, calmodulin-dependent kinase II, calcineurin, and mitogen-activated protein kinases modifies the systolic calcium transient, myofibril function and the activity of transcription factors that coordinate cellular remodeling (67, 68).

Due to its potent effect on the coronary circulation, the interest for the role of ET-1 in the physiology and pathology of this particular vasculature increased rapidly. In the first publication where ET-1 was identified, also it was indicated that this peptide provokes a pronounced constriction of isolated coronary arteries (70, 71), and since this observation most of data show that ET-1 produces pronounced coronary vasoconstriction in vivo (84, 85) and in vitro (70, 71, 86, 87).

In our laboratory we found that ET-1 evokes marked coronary vasoconstriction in vivo and in vitro. In anesthetized goats, i. v. injections of ET-1 increased systemic arterial pressure, did not change resting blood flow in the left anterior descending or left circumflex coronary (LCx) arteries, and in the middle cerebral artery (MCA) arteries, but it increased coronary and cerebral vascular resistance, and this increment was higher in the coronary circulation than in the cerebral circulation. In other group of anesthetized goats, intra-arterial injections of ET-1 decreased the LCx flow more than MCA flow. In isolated segments from large coronary and cerebral arteries from goats, ET-1 caused contraction, and the concentration causing 50% of the maximal effect and the maximal contraction were higher in coronary arteries than in cerebral arteries. The in vitro reactivity of these two types of arteries was unaffected by endothelium removal or by indomethacin. Therefore, ET-1 may produce coronary and cerebral vasoconstriction in vivo and in vitro, probably by acting directly on vascular musculature. The results from this study suggest that the sensitivity to this peptide is higher in isolated cerebral arteries than in isolated coronary arteries, but the reactivity in vivo could be higher in the coronary circulation than in the cerebral circulation (88). The coronary vasoconstrictor effect of ET-1 was also observed in isolated, perfused hearts from rats (89), and in isolated coronary arteries from pigs (90). Figure 5 shows actual recordings of the coronary effects of ET-1 in one anesthetized goat and in one isolated, perfused rat heart.

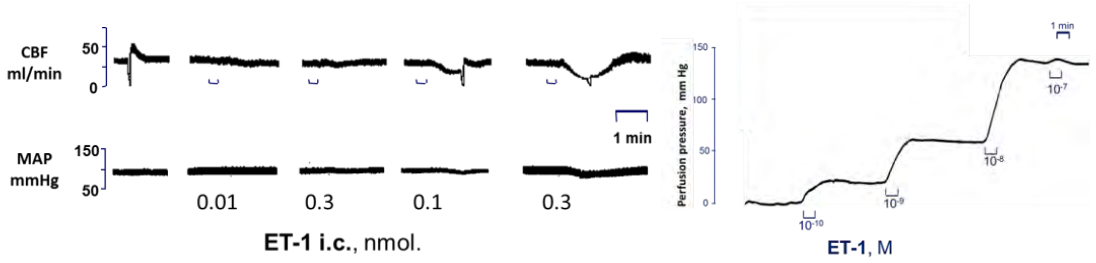

Figure 5. Actual recordings of the coronary effects of ET-1obtained in one anesthetized goat (left) and in one isolated, perfused rat heart (right); I. c. =intracoronary.

There are studies suggesting that the coronary vasoconstriction by ET-1 is mediated by ETA receptors in humans (91), pigs (92) and rats (93), and that the role of ETB receptors is not clear, although it may be of less significance (91). Balwierczak (94) reports data suggesting that coronary response to ET-1 is mediated by both ETA and ETB receptors. With regard to the role of NO, it has been suggested that ET-1 can release NO in the coronary vasculature, and that this NO induces vasodilatation (92, 95) and counteracts the vasoconstriction produced by ET-1 (93). Regard to the role of prostanoids, the results reported are contradictory (93, 95). In our laboratory, we observed that ET-1, intracoronarily injected, produced marked reductions in coronary blood flow, and that these effects were diminished by BQ-123, specific antagonist for endothelin ETA receptors, but not during the infusion of BQ-788, specific antagonist for endothelin ETB receptors. IRL 1620, specific agonist for endothelin ETB receptors, intracoronarily injected, slightly reduced basal coronary blood flow. The effects of IRL 1620 were not modified by BQ-123 or BQ-788. With L-NAME, the reductions of coronary blood flow by ET-1 were potentiated and those by IRL 1620 were not changed. Meclofenamate (cyclooxygenase inhibitor) modified neither the basal values of hemodynamic variables nor the coronary effects of ET-1 and IRL 1620. Therefore, ET-1 produces pronounced coronary vasoconstriction, which may be mediated by endothelin ETA receptors, with no participation of endothelin ETB receptors; NO, but not prostanoids, may inhibit ET-1-induced coronary vasoconstriction. Also, it is suggested that the coronary vasoconstriction by ET-1 may impair cardiac performance due to heart ischemia (96). From the studies with ET-1 and L-NAME in isolated, perfused hearts from rats (89) and in isolated coronary arteries from pigs (90) similar conclusions can be obtained about the modulatory role of NO in the coronary effects of ET-1 (see below Ischemia-reperfusion). Figure 6 shows an estimation of possible interaction between NO and ET-1 in the coronary circulation derived from the observed effects of inhibition of NO synthesis with L-NAME on the coronary response to ET-1 in anesthetized goats (96), isolated hearts from rats (89) and isolated coronary arteries from pigs (90).

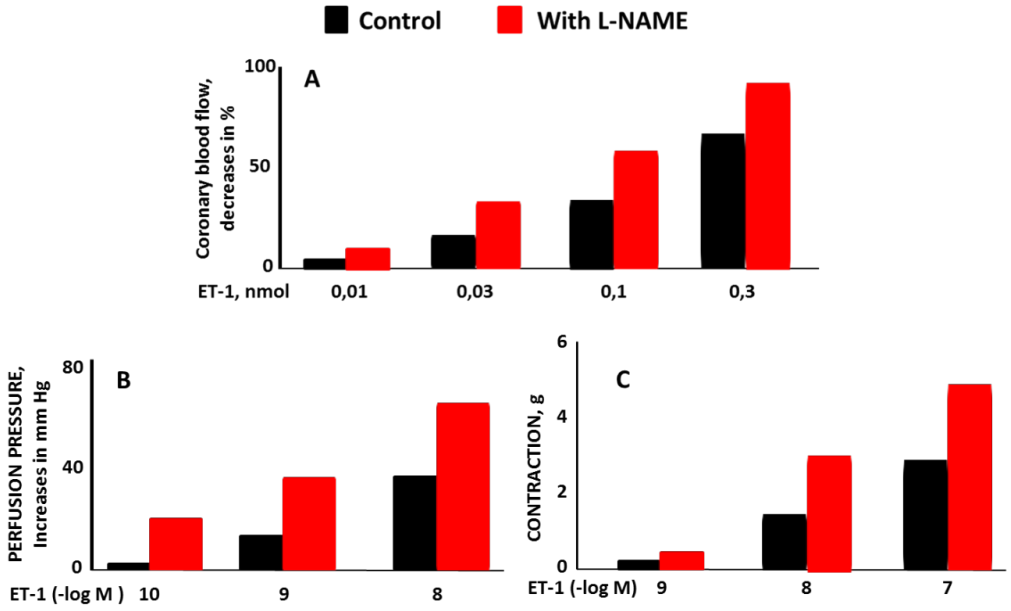

Figure 6. Summary of the effects of inhibition of NO synthesis with L-NAME on the action of ET-1 in the coronary circulation of anesthetized goats (A) and of isolated, perfused hearts from rats (B), as well as in isolated coronary arteries from pigs (C). The augmentation of the coronary response to ET-1 was qualitatively similar in the three experimental preparations.

ET-1 may also exert other vascular functions, as for example to modulate the response of coronary arteries to other types of vasoactive stimuli. The relaxation to isoproterenol, field electrical stimulation, Ach, and sodium nitroprusside was recorded in isolated coronary arteries from rats, in the absence and in the presence of ET-1. It was found that the treatment with ET-1 increased the relaxation to isoproterenol and did not modify the relaxation to electrical stimulation, Ach, or sodium nitroprusside The increased relaxation to isoproterenol in the presence of ET-1 was also present in the presence of the endothelin ETB antagonist BQ788 but disappeared in the presence of the endothelin ETA antagonist BQ123 or the blocker of protein kinase C chelerythrine. Thus, curiously, ET-1 may potentiate coronary beta-adrenergic vasodilatation, due at least in part to stimulation of endothelin ETA receptors and activation of protein kinase C (97).

Arginine-vasopressin (AVP) is a hormone that may be of significance in the regulation of coronary circulation, particularly under certain pathological conditions such as myocardial infartion (see below Ischemia-reperfusion). In anesthetized goats we have found that under normal conditions, AVP produces coronary vasoconstriction and that this vasoconstriction may be mediated by vasopressin V1 receptors, without involvement of vasopressin V2 receptors; and it is probably modulated by NO, but not by prostanoids (98). These results in coronary vasculature of goats are in line with those obtained in coronary vasculature from rabbits (99). In one study performed in arteries taken from 7 vascular beds of rabbits, we found that in isolated arteries, AVP induced contraction in central ear (cutaneous), basilar (pial), renal, coronary and saphenous (muscular) arteries, but had no effect in mesenteric and pulmonary arteries; the order of potency for the contraction to this peptide was: ear > basilar > renal > coronary > saphenous arteries. Treatment with L-NAME increased the contraction to AVP in ear (148% of control), basilar (150% of control), renal (304% of control), coronary (437% of control) and saphenous (235% of control) arteries. Removal of the endothelium increased the contraction to AVP in basilar (138% of control), renal (253% of control), coronary (637% of control) and saphenous (662% of control) arteries, but not in ear artery. Mesenteric and pulmonary arteries in the presence of L-NAME or after endothelium removal did not respond to AVP, as occurred in control conditions. The specific blockade of V1 vasopressin receptors was more potent than the blockade of both V1 and V2 vasopressin receptors to block the contraction to AVP. In arteries precontracted with endothelin-1, vasopressin or desmopressin did not produce relaxation. Therefore, these results suggest: a) most arterial beds studied (5 of 7) exhibit contraction to AVP with different intensity; b) the vasoconstriction to this peptide is mediated mainly by stimulation of V1 vasopressin receptors, and c) endothelial NO may inhibit the vasoconstriction to this peptide, especially in coronary and renal vasculatures (99). From this study (99) it is apparent that NO plays a relevant role in modulating the vascular effects of AVP, and that this role is relatively more pronounced in coronary vasculature than in other vasculatures. In other study we compared the coronary effects of ET-1 and AVP in anesthetized goats, and the results suggest that ET-1 is more effective than AVP for producing coronary vasoconstriction (Figure 7), that NO may play a more relevant role for modulating the coronary vasoconstriction provoked by ET-1 than by AVP (Figure 7), and that cyclooxygenase products may not be involved in the coronary effects of these two peptides (100). From these results it can be estimated that after inhibition of NO synthesis with L-NAME, the increase in coronary vasoconstrictor effects produced by ET-1 is about two times higher than those induced by AVP (Figure7).

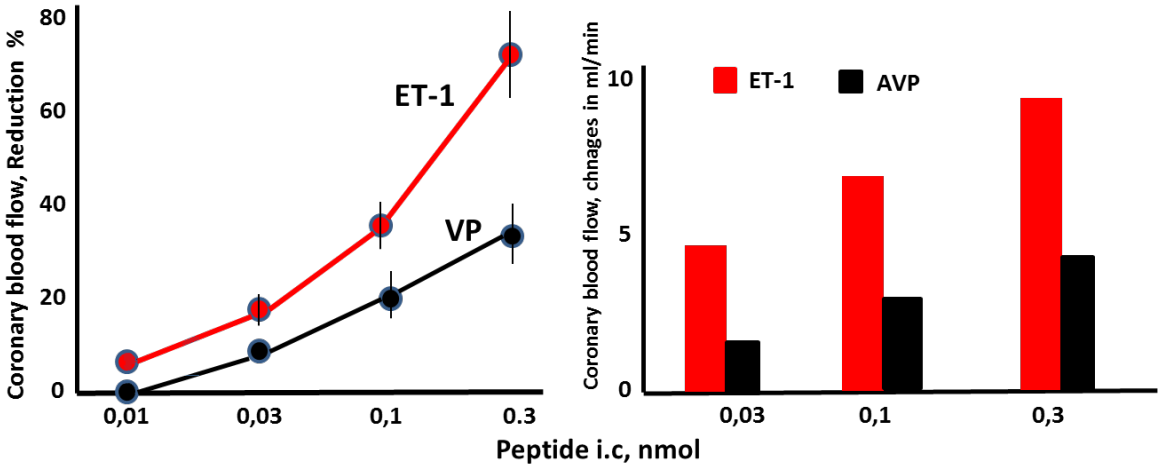

Figure 7. Comparison of the coronary vasoconstrictor effects of ET-1and AVP (left), and of the relative role of NO in modulation of these effects (right). These studies were performed in anesthetized goats and both peptides were injected intracoronarily (i. c.).

2.5. Interaction between NO and ET-1

The functional interaction between NO/ET-1 deserves attention for its implication in the regulation of vascular function under normal and some pathological conditions. The release of ET-1 is inhibited by NO from the endothelium (83, 101), which make NO and ET-1 functionally closely interdependent, with a strong inhibitory effects of ET-1 on NO-mediated dilation, and vice versa (102-104). Endothelial dysfunction is associated with a decline in the contribution of NO in favor of greater influence of ET-1 on vascular function, and this phenomenon seems to be more evident in the coronary circulation under certain pathological conditions (80). This alteration in the balance between NO and ET-1 under certain conditions may be one of the aspects underly dysfunction of the coronary circulation with deleterious consequences for the myocardium (78, 80).

Alterations in the NO/ET-1axis can lead to disbalance in vascular endothelial function, resulting in pathological changes (105). With regard to this, it has been demonstrated that intracoronary infusion of BQ123 resulted in beneficial effects on coronary diameter and coronary blood flow in patients with coronary artery disease (106). Other observations also support the idea that in patients with coronary artery disease, less NO is produced and more ET-1 would contribute to vascular tone (80), and this hypothesis was tested in patients with coronary artery disease where it was proved that ET-1, via endothelin ETA receptors, contributed to the reduction of endothelial dilatory response (107). Other study in patients with coronary artery disease shows that blockade of endothelin ETA receptors or blockade of both endothelin ETA and ETB receptors improved the endothelium-dependent dilation in coronary arteries (108). Data from other studies also suggest that when more severe is atherosclerosis in coronary arteries, more importance has ET-1 in the control of coronary artery tone, thus functional contribution of this peptide appears to rise with the severity of coronary artery disease (80).

Other circumstance in which interaction between NO and ET-1 may be altered is during aging. This condition may be associated with alterations in the cardiovascular system, and with increased ET-1 plasma levels. From a study performed in hearts from young (3 months old), aged (24 months old) and aged rats after 3 months of caloric restriction, it is suggested that aging is accompanied by alterations in myocardial and coronary responses to ET-1, that may be related to changes in expression of NO synthases and/or endothelin receptor subtypes, with some of these changes being prevented by caloric restriction (109).

The relation between NO and ET-1 may be also altered during hypotension. In anesthetized goats, during acute hypotension induced by constriction of the caudal vena cava, it was found that: 1) under normotension, ET-1 and AVP, intracoronarily injected, decreased coronary vascular consuctance (CVC) by up to 56% for ET-1 and 40% for AVP; 2) under non-treated hypotension, the decreases in CVC byET-1 were augmented approximately 1. 5 fold, and those by AVP were not modified; 3) this increase in CVC by ET-1 was not affected by L-NAME and was reversed by meclofenamate, and 4) the coronary effects of AVP were not modified by any of these treatments. Therefore, acute hypotension increases the coronary vasoconstriction in response to ET-1 but not in response to AVP, and this increased response to ET-1 may be related to both inhibition of NO release and release of vasoconstrictor prostanoids (110).

The data exposed above suggest that NO and ET-1 play a relevant role in the regulation of the coronary circulation, and that a functional intereration between these two substances could be of relevance in the control coronary vacular tone. When it is compared the role of NO in the coronary effects of ET-1 and AVP, the data suggest that the interaction between NO and ET-1 is greater that the interaction between NOand AVP in the regulation of the coronary circulation (see Figure 7).

Summarizing, the alteration in NO/ET-1 axis may play a relevant role on pathogenesis of coronary artery disease, and thisaxismay be a target for therapeutic interventions in some pathological conditions (e. g., atherosclerosis, myocardial ischemia-reperfusion) (Figure 8).

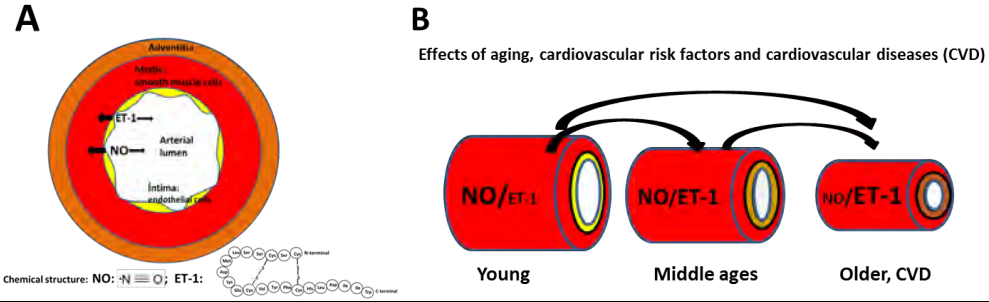

Figure 8. A) Schematic representation of a transverse section of a normal artery showing the layers of the artery wall, the release of NO and ET-1 by endothelial cells, and chemical structure of these two substances. B) Schematic representation of changes in the relative role of NO and ET-1 in the regulation of vascular tone after aging, cardiovascular risk factors and some cardiovascular diseases (CVD).

3. ISCHEMIA AND REPERFUSION

Sudden interruption of blood supply through a coronary artery can occur in patients as a result of the rupture or erosion of an atherosclerotic plaque situated in the wall of a coronary artery, with a superimposed thrombosis of this artery. Sudden interruption of blood supply through a coronary artery also can be reproduced in laboratory animals in which mechanical occlusion of a coronary artery can be induced. In both cases, coronary occlusion results in myocardial infarction if coronary perfusion is not restored within <40 min. Following a lag phase of fully reversible damage, myocardial infarction spreads depending on duration of coronary occlusion as a wave front phenomenon from the most central core of the affected coronary perfusion territory to its peripheral borders. The main determinants of the final amount of myocardium affected (size of the myocardial infarction) are the coronary circulation (area at risk), duration of coronary occlusion and presence of collateral blood flow. The area at risk is the anatomic perfusion territory of the coronary artery distal to the site of its occlusion, and this area determines myocardial infarct size, and infarct size is a principal determinant of prognosis of patients. The duration of coronary occlusion is determined both by the initial occlusive event and the restoration of coronary perfusion, and this restoration may be spontaneous or through a therapeutic intervention. In healthy and young experimental animals, the coronary circulation may be not compromised by atheromathosis and the onset and release of the mechanical coronary occlusion are clearly defined. In patients, however, the situation is very different and more complex, as coronary vasculature is usually deseased, and complete coronary occlusion may be preceded by intermittent changes in blood flow. In addition to the area at risk and the duration of coronary occlusion, residual blood flow through the coronary collateral circulation may be a significant determinant of infarct size. Studies in experimental animals have been performed in species with and without an innate coronary collateral circulation, and therefore the conditions differ. In humans, that have an innate coronary collateral circulation, the possibility of a recruitable collateral circulation may improve the outcome of patients with coronary artery disease (for details, see Reference 14). Questions that could be of interest about this particular issue are: damage of coronary vasculature and of cardiomyocytes develops in parallel during ischemia-reperfusion? Damage of coronary vasculature and of cardiomyocytes influences each other during this condition?

In relation to the effects of ischemia and reperfusion on the heart, there are recent excellent revisions (9, 13, 14, 111-114), and these effects are summarized below.

3.1. Effects of ischemia

Coronary occlusion results in abrupt deprivation of oxygen supply to the myocardium, which induces ischemia and, it left uncorrected, death of myocytes by necrosis. Cessation of oxygen supply disrupts aerobic metabolism and oxidative phosphorylation with the consequent depletion of ATP and other energy compounds, and increased concentration of lactate and acidosis in cardiomyocytes. Depletion of energy compounds rapidly causes myocardial dysfunction and detrimental effects on biochemistry and metabolism of cardiomyocytes. These effects of oxygen deprivation, however, can be reversed if the duration of ischemia lasts <20 min. But if ischemia continues, irreversible injury develops, which is characterized by disruption of plasmatic, sarcolemmal and lisosomal membranes of the cardiomyocytes. This induces loss of osmotic balance, alteration of ions exchange through membranes and leakage of cellular metabolites into the extracellular space, with increased concentration and overload of calcium inside of cells and remaining closed the mitochondrial permeability transition pore. Damage of mitochondrial membranes reduces the ability of cells to produce ATP upon reperfusion, as well as the release of digestive enzymes, culminating in cell necrosis and apoptosis (13, 111-114).

The consequences of ischemia depend on several factors, as for example the duration of the occlusion (115), whether this ischemia is partial or global, and the presence or not of functional collateral circulation (116). Shrader et al. observed a reduction of 65% in ATP after 15 minutes ischemia (117), and Jones et al. in a murine model observed a reduction of 95% in ATP after 40 minutes ischemia (118). At the beginning of ischemia, the oxidative phosphorylation stops to work, and there is a transition from aerobic to anaerobic respiration, which may be detectable by observation of the electrocardiographic modifications (119) and the decrease of the myocardial contractility (120). In the myocardium, the activated glycogenolysis is quickly slowed down and the intracellular pH may decrease to 6. 2 after 10 min (121). The cause of the decrease in pH during ischemia is not clear (122, 123).

3.2. Effects of reperfusion

In the 1960s, R. B. Jennings and cols. (12) and R. A. Krug et al. (124) published that reperfusion by itself can damage the myocardium previously exposed to ischemia, and advanced the pioneering concept of “reperfusion injury” (13). These authors showed that if reperfusion is applied within 20 min after provoking coronary occlusion, myocardial affected is recovered and did not die. When ischemia was prolongedto20-40 min before institution of reperfusion, subendocardial myocardial cells underwent necrosis, but midmyocardial and subepicardial cells were salvaged. When duration of ischemia extended from 40 min to 3 h, necrosis affected in ischemic risk zone from the subendocardium to the subepicardium. During this ischemia, it develops the phenomenon known as “wave front phenomenon of ischemic cell death” (13, 111-114, 125, 126). These ideas

are the basis of the current therapy for patients that suffer AMI (Figure 9).

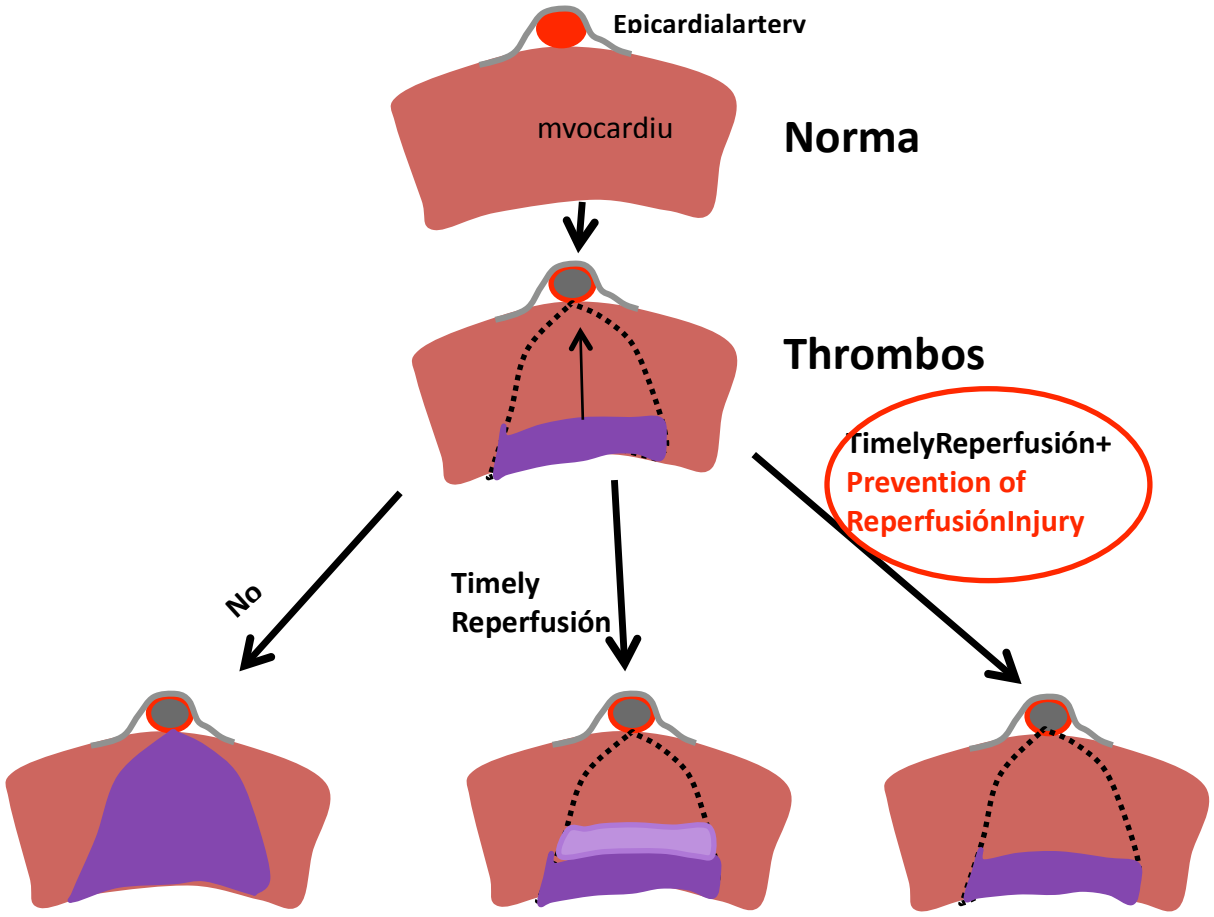

Figure 9. Schematic representation of a fragment of myocardium and an epicardial coronary artery under normal conditions and after artery thrombosis with theoretical evolution of myocardium necrosis, non-exposed and exposed to reperfusion. RI=reperfusion injury. Acute myocardial infarction is a dynamic process that frequently reaches the total extension of myocardium necrotized within 6 h after initiating the artery occlusion.

Restauration of blood supply to the zone of the myocardium exposed previously to ischemia (reperfusion) have beneficial effects and also adverse effects in the myocardium, by which Braunwald and Kloner wrote in 1985 that reperfusion by itself may be a "double-edge sword" (11). Reperfusion injury refers to myocardial, vascular, and/or electrophysiological dysfunction that is induced by the restoration of blood flow to previously ischemic myocardial tissue. It is recognized four forms of reperfusion injury, of which two may be reversible and other two may be irreversible (111):

1) Reversible forms: a) cardiac arrhythmias, particularly ventricular arrhythmias, which usually self-terminate or are easily treated (125), and b) myocardial stunning, with contractile ventricular dysfunction as consequence of alteration of myocardial contractile apparatus (128).

2) Irreversible forms: a) m*icrovascular plugging*, first described by Krug et al. in 1966 (124). It may be a consequence of capillary damage, endothelial and cardiomyocyte cells swelling, micro-embolization of material released from the atherosclerotic plaque, micro-thrombi, release of vasomotor and thrombogenic substances, and neutrophil cumulation (129, 130). These alterations may be related, at least in part, to the non-reflow phenomenon, and b) *lethal myocardial reperfusion injury*, characterized by death and apoptosis of cardiomyocytes that were viable when reperfusion is applied (131, 132 http://www. jci. org/articles/view/62874 - B2). Lethal myocardial reperfusion injury may account for up to 50% of the final myocardial infarction size, and this type of injuries attenuates the benefits of myocardial reperfusion, and

thus represents a target for cardioprotection. At present, no effective therapy exists for this situation. It has been estimated that timely (early) reperfusion can salvage approximately 50% of severely ischemic myocardial and that prevention of myocardial reperfusion injury could prevent the necrosis of an additional 40% (111).

Most of studies suggest that the major factors contribute to myocardial reperfusion injury are intracellular calcium overload, overproduction of radical oxygen species, abrupt restoration of pH in ischemic zone, and hipercontracture (9, 13, 111, 132). These phenomena have been involved in opening a channel in the inner mitochondrial membrane (mitochondrial permeability transition pore), which permits the entry of calcium and radical oxygen species into mitochondria, leading to an energetic failure, and death and apoptosis of cardiomyocytes. Sarcolemmal disruption may be the feature that causes irreversibility, but its pathogenesis is at present unknown (9, 13, 111, 132). Figure 10 summarizes the main intracellular factors involved in the effects of ischemia and of reperfusion in the cardiomyocytes affected by coronary artery occlusion.

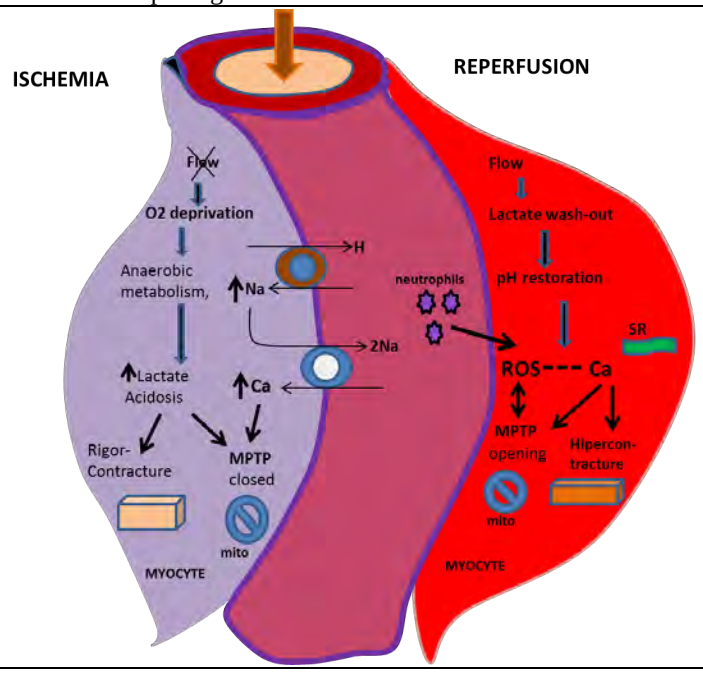

Figure 10. Schematic representation summarizing main intracellular changes that may occur in cardiomyocytes after interruption of blood supply to a zone of myocardium (Ischemia, left), and after restoration of blood supply to this myocardial ischemic zone (Reperfusion, right).

The available data indicate that not only cardiomyocytes but also the coronary vasculature (with its endothelium) is damaged after coronary ischemia-reperfusion. In this condition, the coronary circulation deserves attention because of it occupies a central position in the cause and consequences of ischemia-reperfusion, and it can be damaged in different degrees. This damage may vary from moderate impairment of endothelium function to severe both functional and structural alterations with edema of the vascular wall, and ultimately to vascular obstruction and the no-reflow phenomenon. These alterations are involved in dysregulation of coronary blood flow and functional ability of coronary collaterals, and therefore in evolution of ischemia-reperfusion and in pathophysiology of reperfusion injury. In consequence, the study of coronary vascular and endothelial function after ischemia-reperfusion is a focus of interest for investigators to understand pathophysiology of reperfusion injury, and to improve therapeutical strategies for this condition.

Several factors, including dysregulation of coronary function, increased coronary vascular resistance and microvascular obstruction are detrimental for coronary perfusion after ischemia-reperfusion. Underlying these alterations may be disbalance between endothelial vasodilators and vasoconstrictors, coronary microembolization, inflammatory cell infiltration and post-ischemic hyperpermeability. The coronary endothelium is one of the victims of ischemia-reperfusion. Since the observation by D. D. Ku in 1982 (133) it is known that ischemia-reperfusion induces endothelial cell dysfunction, with impairment of endothelium-dependent coronary vasodilatation and augmented coronary reactivity to endothelium-modulated vasoconstrictors. Also, it seems to be that the endothelial function may be very sensitive to ischemia-reperfusion as it has been observed that after brief (15 min) ischemia, coronary vasodilator response to Ach was impaired during the first hour of reperfusion and gradually improved over a 90-minute, with no evidence of endothelium structural damage; in this same study, it was found that 20 and 30 minutes of ischemia completely impaired endothelium-dependent vasodilation, and induced endothelium structural damage (134). Many

subsequent studies have confirmed these observations, as brief periods of ischemia, including partial ischemia, impairs coronary vasodilator responses and enhances coronary vasoconstrictor responses, and that these alterations are accentuated after prolonged periods of ischemia accompanied by myocardial infarction, and they affect both epicardial and microvascular coronary functional ability (135-140). The mediators of the impairment in coronary vasomotor function after ischemia-reperfusion remain uncertain; it may be involved reactive oxygen species (138), plaque rupture with embolization of particulate debris and release of vasoconstrictors (serotonin, thromboxane (141, 142)), and inflammatory mediators, particularly TNFα (143, 144).

More recent studies support the idea that NO and ET-1 play a relevant role at various subclinical and clinical stages of the coronary artery disease, and particularly after myocardial ischemia-reperfusion. The NO/ET-1 axis could play an important role in pathophysiology of myocardial ischemia-reperfusion and of reperfusion injury, and beneficial effects of some therapeutical strategies for myocardial reperfusion injury are attributed to modification of this axis. This particular issue, however, remains to be resolved and the available results are contradictory. As NO and ET-1 may interact for regulating the function of coronary vasculature and the myocardium, the study of these two substances probably should be considered together. NO is produced by both coronary endothelial cells and cardiomyocytes, and the principal source of this NO is eNOS (NOS3); in cardiomyocites NOS1 may be also involved, and expression of both NOS1 and NOS3 in these cells appears to be species-dependent (for this issue, see References 36, 145-148). On the other hand, NO seems to play a relevant role in the functional regulation of myocardium under normal conditions and after ischemia-reperfusion (36, 147-149). Several studies demonstrate that biosynthesis of NO by constitutive NOS (endogenous NO) plays a critical role in alleviating the severity of myocardial ischemia-reperfusion as well as reperfusion injury. NO has been known to play various functional and pathological roles as an intracellular or intercellular messenger in the heart, and there are data indicating that NO can both reduce and mediate myocardial reperfusion injury. NO influences myocardial perfusion by regulating coronary vascular function, causing a direct vasodilatory effect and modulating vascular reactivity to other types of vasoactive stimuli (e. g. ET-1). NO can also influence indirectly myocardial perfusion by regulating leukocyte–endothelial cell interaction, inhibiting platelet adhesion and aggregation, attenuating smooth muscle cell proliferation, and possible modulation of cardiomyocytes function. NO released from the endothelium has also been shown to inhibit surface expression of many endothelial cell adhesion molecules (ECAMs), including P-selectin, E-selectin, VCAM-1, and ICAM-1 (for this issue, see Reference147). Also, as cardiomyocytes are surrounded by capillary endothelial cells it allows for cell-to-cell signaling between these two types of cells. Interactions between endothelial cells and cardiomyocytes could be of significance in regulating cardiac function by modulating vascular tone and by stimulating proliferation of neighboring cells. This aspect may be of relevance in situations such as ischemia and reperfusion (149).

There are studies showing that NO may be detrimental, and also studies showing that NO may be critical to preserve cardiomyocytes function and viability after ischemia-reperfusion. In a Review published by R. Bolli in 2001 it is indicated that “Of the 92 studies that have examined the role of NO in modulating the severity of ischemia-reperfusion injury in unstressed myocardium, the vast majority (73%) have concluded that NO (either endogenous or exogenous) has a protective effect, and only 12% found a detrimental effect. The proportion of studies supporting a citoprotective role of NO is similar for in vivo and in vitro preparations” (150). The cardiac interstitial NO concentration may increase during early ischemia and early reperfusion, and this increase may be in part derived from activated NOS isoforms but also from NOS-independent pathways. The cardiac interstitial NO concentration is within the nanomolar range during normal perfusion. And during ischemia, NOS3 activity is increased within minutes, and subsequently the NO concentration during early ischemia is increased (151). However, with prolonged myocardial ischemia, NOS3 protein expression decreases, and the tissue acidosis attenuates NOS3 activity (153). Within the early seconds of reperfusion, the NO concentration is increased (154), and if reperfusion is prolonged, NOS activity and thus NO concentration decrease below baseline values (155). Decreased NO production during reperfusion have been suggested by observations indicating loss of NO-dependent vasodilation in response to Achor bradykinin, or loss of vasoconstriction in response to NOS inhibition (for details, see Reference 146). During myocardial ischemia-reperfusion, reduced production of endogenous NO could leave unopposed coronary vasoconstriction to the presence of vasoconstrictors such as ET-1. This disbalance between NO and ET-1 in turn enhances vascular tone, decreases coronary blood flow and exacerbates reperfusion injury.

From the results obtained in a study performed in rat hearts, it is suggested that during reperfusion, cardiac function is depressed, despite increased rather than decreased endogenous NO production, largely due to the prevalence of the deleterious effects of ET-1. These adverse effects of ET-1 can be overcome by antagonism of ET-1 receptors or exogenous NO supplied by NO donors (156). In other study performed in anesthetized pigs (157), it was examined the interaction between the cardioprotective effect of endothelin receptor blockade and NO during ischemia-reperfusion injury. The authors of this study suggest that blockade of endothelin ETA receptors produces cardioprotective effects, and that this cardioprotection is mediated via a mechanism related to NO (157).

In a Review published by R. Schulz et al. in 2004 (146), the authors remark the importance of endogenous and exogenous NO when given at the time of reperfusion for vascular and myocardial function, and morphological outcome following ischemia-reperfusion. Administration of NO, NO donors or drugs that enhance NO release (statins, calcium antagonists, angiotensin-converting-enzyme-inhibitors, dexamethasone) prior to ischemia protects the myocardium against ischemia-reperfusion injury. This exogenous administration of NO prior to ischemia can initiate a preconditioning-like phenomenon. Interestingly, in this study it is suggested that endogenous NO derived from NO-synthase is not involved in triggering or mediating the early phase of ischemic preconditioning's protection, but it does play a pivotal role for initiating and mediating the delayed phase of ischemic preconditioning's protection (146).

The variation in plasma levels of several factors, including ET-1 and NO, was examined in a rabbit model of acute myocardial ischemia-reperfusion (158). Rabbits were exposed to open-chest surgery and were separated in three groups: group A received sham-surgery, group B was the reperfusion group, and group C was the infarction group. At 12 h and at 24 h after chest closure, plasma levels of ET-1 in groups B and C were higher than before chest surgery, and at 24 h there was no significant difference between these two groups. NO levels in groups B and C at 12 h after chest closure were decreased compared to those before chest surgery. NO levels in group B at 24, 48, and 72 h were lower than those at 12 h, while those of group C were not significantly changed after 12 h. These results show that ET-1 and NO are mutually antagonistic vasoactive substances, and that after myocardial ischemia-reperfusion, sustained reduction of NO may occur, thus suggesting that NO supplement is a good clinical choice. Dynamic monitoring and comparison of plasma levels of ET-1 and NO, as well as of other factors, revealed that appropriate intervention of these factors may reduce reperfusion injury. The authors of this same study suggest that plasma ET-1 levels may be used as a reference index for the diagnosis and determining the prognosis of myocardial infarction. It seems to be that endogenousET-1 is increasingly released in systemic circulation and coronary circulation, mainly in an autocrine/paracrine manner by endothelial cells. This study of Zhao et al. (158) also showed that 12 h after myocardial ischemia, plasma ET-1 levels increased, while calcitonin gene-related peptide (CGRP) levels decreased. Various factors and metabolites produced during the early phase may have a stimulating effect on endothelial cells, increasing the synthesis and release of ET-1, and leading to both the consumption and the decreased levels of CGRP. From this study (158) it can be inferred that NO mediates reperfusion injury, but it also may reduce reperfusion injury through its antagonism on the effects of ET-1. However, this particular issue requires further investigation.

With regard to mechanisms underly the effects of NO after ischemia-reperfusion, several hypothesis have been proposed. In relation to the possible protective role of NO during ischemia-reperfusion, it has been proposed that it is related to the anti-peroxidation effect (156), as well as to resistance towards neutrophil adhesion and aggregation (159). In relation to NO-mediated myocardial injury, it has been suggested that it may be primarily related to generation of large amounts of oxygen free radicals as consequence of an excess of NO, which reacts rapidly with oxygen (154). These circumstances may lead to reperfusion injury and aggravate the condition (160). A study carried out in cultured cardiomyocytes suggests that the enhanced production of NO was critical in balancing ATP supply and demand during ischemia, and also in protecting cardiomyocytes from ischemia-reperfusion injury (161).

The development of gene-targeted mice has allowed to begin to define the cellular and molecular mechanisms involved in the pathogenesis of acute myocardial infarction, although the results from these experimental models should be taken with caution. The development of mice with genetic manipulations could provide useful information regarding pathologic mechanisms related to myocardial ischemia-reperfusion injury (for details, see Reference 147). Studies using a model of eNOS transgenic mice have demonstrated that eNOS overexpression significantly attenuates the extent of myocardial infarct size following coronary artery ischemia and reperfusion (162).

Summarizing, the studies exposed before suggest that the role of NO in ischemia-reperfusion and reperfusion injury is not clear as there are data suggesting that NO may be beneficial and that it may be detrimental in evolution of myocardial ischemia-reperfusion. As hypothesis, it might be that at the beginning of reperfusion the presence of NO might be beneficial, particularly for coronary vascular vasomotion, but at chronic stage its presence might be pernicious for heart tissue because of it would facilitate formation of free radical species.

With regard to ET-1, many data suggest that this peptide is involved in the pathophysiology of reperfusion injury (67, 68, 163-166), and that it may be also a significant predictor of reperfusion injury (167, 168). During ischemia-reperfusion, the production of ET-1 is increased, the coronary vasoconstrictor effects of this peptide are also increased, and ET-1 enhances myocardial necrosis and arrhythmogenesis (67, 68, 80). Plasma ET-1 levels increase 3–4h after the onset of ST segment elevation myocardial infarction, peak within the first 24h and remain elevated after 48h (168). High ET-1 values after percutaneous coronary intervention have been linked to poor prognosis, including higher 30-day mortality (168). In humans, evidence of microvascular injury has been obtained as angiographic scores, primarily by indirect methods such as the thrombolysis in myocardial infarction grade flow and myocardial blush grade (169) and more recently by cardiac magnetic resonance (170). Results

from patients suggest that high plasma ET-1 levels after myocardial infarction are associated with the presence of microvascular obstruction and lower myocardial salvage index (171).

An interesting study was performed in patients with AMI that underwent reperfusion therapy or conservative drug therapy. Patients who underwent reperfusion therapy had significantly lower eNOS and NO levels, and higher plasma ET-1 levels than those who received conservative drug therapy. All patient groups had significantly lower eNOS and NO levels, and higher ET-1 levels, than healthy controls. There was a significant positive correlation between eNOS and NO, as well as significant negative correlations between eNOS/ET-1 and NO/ET-1 (172). In other study it has been found that the circulating level of ET-1 was considerably higher in the non-spontaneous reperfusion patients than in the spontaneous reperfusion patients; the ET-1 level was the only significant predictor of spontaneous reperfusion, and the ET-1 level at admission is an indicator of spontaneous reperfusion (173). L. Cao et al. (174), after considering observations by others, suggest that ET-1 may act as a negative factor in reperfusion injury. These authors also suggest that ET-1 plays a different role in the different stages of myocardial ischemia-reperfusion, and to ascertain the exact mechanisms, experimental and clinical studies are needed (174). On the other hand, it can not be excluded that after ischemia-reperfusion, ET-1 also may induce beneficial effects on the myocardium. ET-1 could induce cardioprotection against infarct size and ventricular arrhythmias, through as yet incompletely understood mechanisms (175), as well subsequent infarct-healing and early ventricular remodeling (166, 176).

Data from literature suggest that ET-1 plays a relevant role in development of coronary artery disease and in the effects of myocardial ischemia-reperfusion (Figure 11).

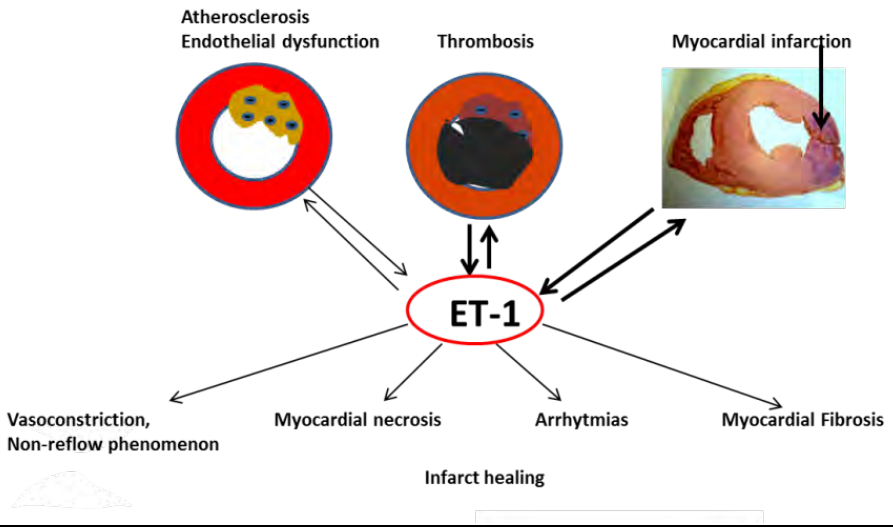

Figure 11. Schematic representation of the probable role of ET-1 in the pathophysiology of coronary artery disease, myocardial infarction, and heart failure. At preclinical stages, ET-1 could contribute to endothelial cell dysfunction and to atherosclerosis. During acute coronary syndromes, ET-1 could cause adverse effects (vasoconstriction, inflammation, myocardial necrosis, arrhythmogenesis), and during chronic evolution it might have beneficial effects by participating in infarct healing.

The study of the effects of ET-1 and its interaction with NO in coronary vasculature after ischemia-reperfusion may contribute to know its pathophysiology. Respect to this issue, most of studies have been related with the analysis of the effects of total ischemia followed by reperfusion, and very few studies have been performed about the effects of partial ischemia alone or followed by reperfusion. The clinical situation of partial ischemia of the myocardium may be present in patients with coronary atheromatosis, dynamic vasospasm, or transient arterial hypotension.

During reperfusion after total (134) or partial (140) coronary occlusion, endothelium-dependent coronary vasodilatation is decreased. Also, basal release of NO from isolated, perfused rat hearts may be diminished after global ischemia-reperfusion, with implication of the NO pathway (177). On the hand, administrations of exogenous NO diminish the adverse effects of ischemia-reperfusion (178). Other studies, however, showed that inhibitions of NO synthesis may protect, rather than aggravate, the effects of ischemia-reperfusion (18, 179). For ET-1, ischemia-reperfusion can induce increased coronary vasoconstriction in response to this peptide (180) but whether or not this increased effect is present may depend on the severity and duration of ischemia (181). The increased response to ET-1 after ischemia-reperfusion it has been attributed to decreased production of NO and prostacyclin as a result of endothelial dysfunction, and of changes in characteristics of endothelin receptors in coronary vessels (180, 182). The coronary reactivity to ET-1 after ischemia-reperfusion has been explored mainly using in vivo and in vitro preparations, and coronary ischemia for different periods has been induced in vivo by occluding one coronary artery and in isolated, perfused hearts.

In our laboratory, we found in anesthetized goats that during partial occlusion of the left circumflex coronary artery, coronary vascular conductance was reduced by about 30%, and the coronary vasodilatation in response to Achand sodium nitroprusside was decreased in animals non-treated or treated with L-NAME or with meclofenamate; the vasoconstriction in response to ET-1 was depressed in non-treated animals, and this depression was reversed by L-NAME and was accentuated by meclofenamate. At 30 min of reperfusion after this partial coronary occlusion, coronary vascular conductance remained decreased by about 25%, and the vasodilatation in response to Achand sodium nitroprusside, as well as the vasoconstriction with ET-1 were as in the control and were comparable in treated and non-treated animals. These results suggest: a) that during partial ischemia, the coronary vasodilator reserve is greatly reduced, and the vasoconstriction to ET-1 is blunted, with preservation of the modulatory role of NO and involvement of vasoconstrictor prostanoids in this vasoconstriction, and 2) that during its reperfusion, the coronary vasodilator reserve and the coronary reactivity to Ach and ET-1 recover, but the modulatory role of NO in this reactivity may be attenuated (183).

We also explored the effects of reperfusion after short and prolonged, total ischemia on the role of NO and the effects of ET-1 in the coronary circulation. In this case, 15- or 60-min total occlusion of the left circumflex coronary artery was induced in anesthetized goats. In non-treated animals, during reperfusion after 15-min occlusion, the ET-1-induced coronary effects were lightly increased, and the effects of Ach were unchanged. During reperfusion after 60-min occlusion, the ET-1-induced effects were more pronounced, and the effects of Ach were decreased. L-NAME treatment did not modify the coronary effects of ET-1 during reperfusion after both occlusion durations. This treatment inhibited the effects of Ach during reperfusion after 15-min, and after 60-min occlusions. Meclofenamate treatment did not modify the coronary effects of ET-1 and Ach during reperfusion after both occlusion durations. Thus, ischemia-reperfusion could increase the coronary response to ET-1, which is more pronounced during reperfusion after prolonged than after brief ischemia, and that this increased response to ET-1 is probably related to inhibition of NO release, without involvement of prostanoids (184). Figure 12 shows an estimation of the role played by NO in the regulation of the coronary circulation under normal conditions and after ischemia-reperfusion (183, 184).

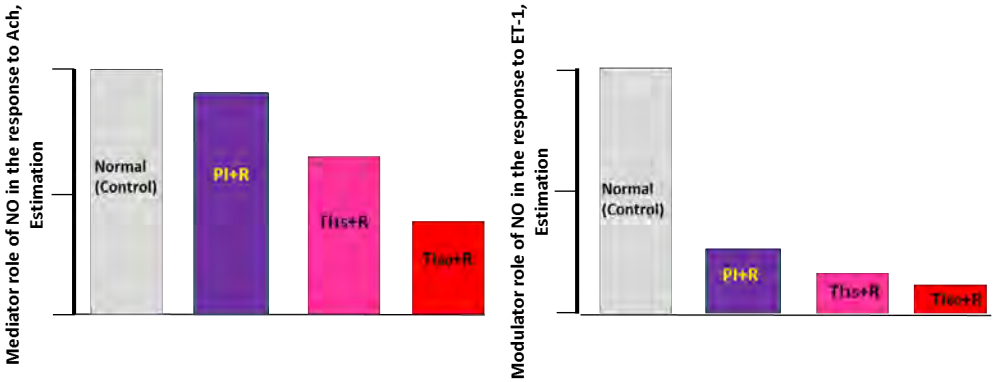

Figure 12. Estimation of the role played by NO in the regulation of the coronary circulation under normal conditions, after partial coronary occlusion followed by reperfusion (PI+R), after 15 min of total coronary occlusion followed by reperfusion (TI15+R) and after 60 min of total coronary occlusion followed by reperfusion (TI60+R). These estimations are based on data obtained in experiments with Ach and ET-1 in anesthetized goats (References 183, 184).

To compare the coronary response to ET-1 with the response to other vasoconstrictor after ischemia-reperfusion, we explored the coronary effects of arginine–vasopressin (AVP). This hormone may be of significance in the regulation of coronary circulation as it can produce coronary vasoconstriction (185), which can be severe enough to cause myocardial ischemia (186). This peptide also could be of interest for understanding the pathophysiology of myocardial ischemia-reperfusion as human plasma levels of AVP are augmented after myocardial infarction (187), and during reperfusion after myocardial infarction (188). Studies performed in dogs show that the coronary effects of AVP are increased in the ischemic myocardium, which can worsen hypoperfusion of collateral-dependent myocardium during exercise (189). Sellke and Quillen (190) report that the coronary action of AVP is augmented after ischemia alone or followed by reperfusion, and the authors suggest that this augmented

effect might be related to alteration of release of NO and prostanoids.

In pages 26 and 27, we have commented two studies from our laboratory where we found that under normal conditions AVP produces marked coronary vasoconstriction and that this vasoconstriction may be mainly mediated by vasopressin V1 receptors, and it is modulated by NO, but not by prostanoids (98). In the other study, we compared the coronary effects of ET-1 and AVP, and the results show that ET-1 produce higher vasoconstrictor effect than AVP. From this study, it is also suggested that NO may play a more relevant role for modulating the coronary vasoconstriction by ET-1 than by AVP, and that cyclooxygenase products may not be involved in the coronary effects of these two peptides (100) (See Figure 7). We then examined the coronary effects of AVP and its interaction with NO and prostanoids during ischemia and reperfusion. During partial coronary occlusion, the coronary vasoconstriction in response to AVP was attenuated in non-treated animals, and this attenuation was reversed by L-NAME and was accentuated by meclofenamate. At 30 min of reperfusion after this partial coronary occlusion, the vasoconstriction with AVP was as in the control and this vasoconstriction was not affected by L-NAME or meclofenamate. These results suggest that: 1) during partial ischemia, the coronary vasoconstriction with AVP is attenuated, with preservation of the modulatory role of NO and probable involvement of vasoconstrictor prostanoids in this vasoconstriction; and 2) during its reperfusion, the coronary reactivity to AVP recover, but the modulatory role of NO in this reactivity may be attenuated. The significance of these results may be that the endothelial function in modulating coronary reactivity to AVP is very sensitive to reperfusion after partial, moderate ischemia, and that AVP may also contribute to the adverse effects of ischemia-reperfusion on coronary vasculature (191). In other series of experiments, we found that at 60 min of reperfusion after 15 min total occlusion of the circumflex coronary artery, the coronary vasoconstriction in response to AVP was increased during reperfusion in non-treated, was not changed in L-NAME-treated and was decreased in meclofenamate-treated animals. Therefore, this study suggests that during reperfusion after a short, total ischemia, the coronary vasoconstriction in response to AVP is increased, probably due to both attenuation of the modulatory role of NO and the release of vasoconstrictor prostanoids (192). This idea is in line with that proposed by others (189, 190), and both of them suggest that AVP should be considered as a factor involved in pathophysiology of myocardial ischemia-reperfusion, although its role could be of less significance than that of ET-1.

We also explored the coronary vasodilator reserve after ischemia-reperfusion examining the hyperemic response after brief coronary occlusions. To this, partial (60 min) or total (15 and 60 min) occlusions of the left circumflex coronary artery were induced, followed in each case by 60-min reperfusion in anesthetized goats, untreated and treated with L-NAME or meclofenamate. Coronary occlusions of 5- and 10-s duration produced hyperemic responses that depended on occlusion duration. After ischemia-reperfusion, these hyperemic responses were diminished, and this diminution was dependent on duration and severity of ischemia. These effects of ischemia-reperfusion were not affected by inhibition of NO synthesis with L-NAME, and were reversed with meclofenamate. The hyperemic response reduction during reperfusion after prolonged ischemia, but not after brief ischemia may be related at least in part to increased production of vasoconstrictor prostanoids (193).

Episodes of myocardial ischemia may occur in humans under situations such as transient arterial hypotension. From experiments in anesthetized dogs it has been reported that the coronary reactivity to ET-1, but not to angiotensin II is increased during low coronary perfusion pressure, which may contribute to coronary ischemia after reduced coronary perfusion (194), and that ET-1 does not modify the coronary response to decreased coronary perfusion pressure after coronary stenosis (195). Hemorrhagic shock in rats is accompanied by decreased perfusion of the heart (and other organs), together with increased ET-1 levels in plasma (196). As a result, the authors suggest that ET-1 is involved in the decreased perfusion of vital and peripheral tissues during hemorrhagic shock (196). Experimental observations have shown that ET-1 (197) and AVP (187) may increase in plasma during some types of hypotension. It seems that under normotension NO may be more relevant in modulating coronary vasoconstriction by ET-1 than by AVP (191), and that NO and ET-1 mutually affect the production and action of each other in the blood vessel wall (198). We performed experiments in anesthetized goats where blood flow through the left circumflex coronary artery was measured electromagnetically, and hypotension was induced by constriction of the caudal vena cava. ET-1 and AVP were directly injected into this coronary artery before (normotension) and during hypotension in animals non-treated and treated with L-NAME, meclofenamate, or both inhibitors. The results of these experiments suggest that hypotension increases the coronary vasoconstriction in response to ET-1 but not to AVP. This increased response to ET-1 may be related to both inhibition of NO release and of vasoconstrictor prostanoids release. This study also suggests that in the coronary circulation, the functional interaction between NO and ET-1 may be greater than that between NO and AVP (199).

To extend our knowledge of the coronary effects of ET-1 after ischemia-reperfusion, the left anterior descending coronary artery of anesthetized pigs was subjected to 30-min occlusion followed by 60-min reperfusion. Then, rings distal (ischemic arteries) and proximal (control arteries) to the artery occlusion were taken from this artery and prepared for isometric tension recording. The sensitivity of the contractile response to

ET-1 and to the endothelin ETB receptor agonist IRL-1620 was augmented in ischemic vessels. The endothelin ETA receptor antagonist BQ-123 decreased the sensitivity of the response to ET-1 similarly in ischemic and control arteries. The endothelin ETB receptor antagonist BQ-788, endothelium removal or L-NAME potentiated the response to ET-1 and to IRL-1620 in control arteries but not in ischemic arteries. Meclofenamate augmented the maximal response to ET-1 in control arteries, and reduced it in ischemic arteries. In precontracted arteries, IRL-1620 relaxed control but not ischemic arteries, and L-NAME or meclofenamate abolished this relaxation. Therefore, ischemia-reperfusion increases the coronary vasoconstriction in response to ET-1 probably due to impairment of endothelin ETB receptor-induced release of NO and prostacyclin, to augmentation of the contractile response to activation of endothelin ETB receptors, and to increased release of vasoconstrictor prostanoids (200). Studies by others were performed in male Sprague-Dawley rats, which were subjected to either heart ischemia–reperfusion (15 min ischemia and 22 h reperfusion), permanent ischemia (22 h) by ligation of the left anterior descending coronary artery, or sham operation (201). Then, the endothelin receptor subtypes mediating vasoconstriction were examined in isolated segments of the left anterior descending and the non-ligated septal coronary arteries. Endothelin ETB receptor-mediated vasoconstriction and receptor protein levels were augmented in coronary arteries exposed to ischemia–reperfusion. In contrast, the ETA receptor-mediated vasoconstriction was unaltered in all groups. It is suggested that ischemia–reperfusion induces local up-regulation of ETB receptors in the smooth muscle cells of coronary arteries in the post-ischemic area. In contrast, in non-ischemic areas, endothelin ETB receptor function was unaltered (201). In a revision published in 2010, Nguyen et al. (80) suggest that the beneficial role of endothelin ETB receptors present under normal conditions may decline with age and risk factors for cardiovascular diseases, revealing smooth muscle ETB-receptors with pro constricting and proinflamatory activities.

We also studied the effects of ischemia-reperfusion on coronary vasculature using isolated, perfused hearts from rats. After 15, 30 or 45 min of global zero-flow ischemia and 15 min reperfusion, the coronary vasoconstriction induced by ET-1 increased after 15 min of ischemia, but not after 30 or 45 min of ischemia. Inhibition of NO synthesis with L-NAME augmented the vasoconstriction induced by ET-1 in non-ischemic hearts, but not following ischemia. These results suggest that in this experimental preparation, ischemia-reperfusion also inhibits NO production, causing an increased coronary response to ET-1 after brief ischemias. Longer ischemias may non-specifically inhibit coronary vasoconstriction and reduce NO production (202). In other series of experiments carried out in isolated, perfused hearts from rats exposed to 30 min global zero-flow ischemia followed by 15 min reperfusion (203), we found that after ischemia-reperfusion, 5-hydroxytryptamine produced contraction in isolated coronary arteries, which was potentiated by treatment with ET-1. This potentiation by ET-1 was lower after ischemia-reperfusion than after control, and the potentiation was reduced by l-NAME, by blockade of endothelin ETA receptors with BQ123 and by blockade of endothelin ETB receptors with BQ788, but not by the cyclooxygenase inhibitor meclofenamate. Thus, ET-1 at low concentrations could potentiate coronary vasoconstriction, and this effect is reduced after ischemia-reperfusion, mediated by both endothelin ETA and ETB receptors and is dependent on NO release. This suggests that ischemia-reperfusion might induce complex effects on the coronary vascular effects of ET-1 (203).

We have also explored the effects of ischemia-reperfusion on the coronary reactivity to other types of substances, the effects of which may be dependent on the endothelium and may be of interest in the context of ischemia-reperfusion. Between these substances are diadenosine polyphosphates (ApnAs), which are molecules that have a chain of 2-6 phosphate groups and they may produce vasodilatation or vasoconstriction of blood vessels depending on the particular ApnA in question. In the coronary circulation, ApnAs produce vasodilation in pigs (204) and in dogs (205) when they are present at nM to mM concentrations, as may exist in plasma under normal conditions. Platelet activation and platelet release of ApnAs, may be involved in the pathophysiology of ischemia-reperfusion (206), and the concentration of ApnAs may increase in coronary venous blood during ischemia-reperfusion (207). As these substances can produce vasodilatation or vasoconstriction depending on the conditions of coronary blood vessels, these compounds could participate in the altered coronary regulation associated with ischemia-reperfusion. Apn5A has a long phosphate chain and it is more likely to produce vasoconstriction and therefore, to be involved in the coronary vasoconstriction that frequently occurs after ischemia–reperfusion. Moreover, the production of Ap5A in the heart is increased during heart ischemia. Compared to control rat hearts, the coronary vasoconstriction to Ap5A was augmented and vasodilation diminished after ischemia–reperfusion. After testing the effects of antagonists for P2 and P2Ypurinoceptors, L-NAME and meclofenamate, it is suggested that ischemia–reperfusion reduces the coronary vasodilatory response and increases the coronary vasoconstriction to Ap5A, due to a reduced influence of purinergic P2Y receptors and/or to the production of vasoconstrictor prostanoids (208). The studies with triphosphate (Ap3A) showed that this substance produces coronary vasodilation in control rat hearts, which was attenuated following ischemia-reperfusion. In this case, the results suggest that the attenuation of the coronary vasodilatation to Ap3A after ischemia-reperfusion could be due to the functional impairment of purinergic P2Y receptors and K (ATP) channels, and/or reduced NO release in this condition (209). Tetrataphosphate (Ap4A), in control rat hearts,

produced vasodilatation, which was reduced by reactive blue 2, glibenclamide, and endothelin-1, but was not affected by L-NAME. After ischemia-reperfusion, the vasodilatation to Ap4A diminished, and in this case the relaxation to Ap4A was not modified by reactive blue 2, L-NAME, glibenclamide, although it was reduced by endothelin-1. These results suggest that the reduction of the coronary relaxation to Ap4A after ischemia-reperfusion may be due to impaired effects of KATP channels and to reduced response of purinergic P2Y receptors (210). These three studies (208-210) also show data suggesting that purinergic receptors may be a way involved in the coronary response to some vasoactive substances and this way may be altered after ischemia-reperfusion. Interestingly, purinergic receptors may be also involved in the coronary vasodilatation in response to Ach after ischemia-reperfusion, but not under control conditions. The role of purinergic receptors in the coronary vasodilation in response to Ach may be developed in coronary vasculature during ischemia-condition in order to compensate the reduction of NO release in this condition (211).

Summarizing, the data exposed before suggest that ischemia-reperfusion alters the regulation of the coronary circulation, which may be induced, in part, by dysfunction/damage of the endothelium. This alteration could lead to modification of the normal balance between NO and ET-1 that exists under normal conditions for the control of coronary vascular tone. After ischemia-reperfusion, the role of NO is reduced, whereas that of ET-1 is augmented, which may have pernicious effects for the functional capacity of coronary vasculature, as for example this vasculature could exhibit an exaggerated vasoconstrictor and inflammatory response, thus contributing to the presence of the non-reflow phenomenon. Therefore, the damage of the endothelium, with the consequent alteration in the interaction between NO and ET-1 may play a crucial role in pathophysiology of disregulation of coronary circulation and of the non-reflow phenomenon after ischemia-reperfusion, and in pathophysiology of reperfusion injury. Also, these two substances by forming a functional axis that acts on the coronary artery wall, probably should be considered together in analyzing pathophysiology of myocardial ischemia-reperfusion and reperfusion injury, as well as in therapeutical approaches for mitigating reperfusion injury.

4. THERAPEUTICAL APPROACHES FOR REPERFUSION INJURY

In an elegant article, E. Braunwald published that during the last 100 years, approximately, the management of AMI has gone through fourth major phases (9). These phases are summarized as follows: The past: a) phase I: the management was limited to bed rest, morphine for pain, as well as digitalis, caloric and fluid restriction, and expectant treatment; b) phase 2: it began in 1961 with a paper by Desmond Julian, where he described what later would be known as the Coronary Care Unit (212). In these Units the patients were monitorized, and they were attendant by training of medical and nursing staff, providing trained nurses with the authority and responsibility to perform this procedure, including external defibrillation, in the absence of a physician. Phase 3 (*The Present*): It is the phase of myocardial reperfusion which was initiated in 1975 by Chazov et al. who lysed coronary thrombi by infusing streptokinase directly into the blocked coronary arteries of patients with AMI (6), and it was demonstrated that timely reperfusion actually salvaged ischemic myocardium (213). During the last quarter century, myocardial reperfusion has been improved progressively by a number of key steps. However, although myocardial reperfusion represents a major advance in the treatment of myocardial infarction, this procedure is often accompanied by myocardial injury, so that E. Braunwald and R. A. Kloner referred to myocardial reperfusion as ‘a double-edged sword (10). Phase 4 (*The Future*): the prevention of myocardial reperfusion injury. Many interventions to prevent or diminish myocardial reperfusion injury have been studied (214). It has been estimated that timely, adequate reperfusion can salvage approximately 50% of severely ischemic myocardium (215), and that prevention of myocardial reperfusion injury should prevent the necrosis of an additional 40% (112). Nevertheless, further experimental and clinical research on myocardial reperfusion injury should be carried out (9).

The prognosis of AMI largely depends on its extent, and the final necrotic extent is mostly due to the speed of the progression of ischemic injury and the duration of the ischemia (215, 216). The most effective treatment to limit infarct size is early reperfusion, and the window in which reperfusion effectively limits infarct size in patients with ST segment elevation myocardial infarction is short. After 3 h of ischemia, without collateral circulation and residual flow, the amount of salvaged myocardium is small or nonexistent (217). Nonetheless, late reperfusion may be also beneficial, and should generally be performed within 12 h of symptoms onset (218). In this case, however, the benefit is due to the positive effects of reperfusion on healing of myocardium damaged (218).

Protect patients against myocardial damage caused by ischemia-reperfusion has became a public health problem. Because the best strategies against the morbidity and mortality of AMI is to minimize the ischemic death of myocardial tissue, there are a notable interest for investigating and learning how the heart can be protected from ischemic death. The development of new approaches, including new techniques, as well as the discovery of novel drugs could provide means for new ways of heart protection against ischemic myocardial injury. About this particular issue, several and revisions are available (9, 122-124, 217-220).

A number of therapeutic strategies for preventing myocardial reperfusion injury have been developed. In general, they can be grouped in two ways: a) non-pharmacological strategies (conditioning phenomena: ischemic preconditioning (IPC), ischemic postconditioning

(IPOC), and remote ischemic preconditioning (RIPC)); and b) pharmacological strategies (use of drugs).

4.1. Conditioning phenomena

IPC: Observations showed that reperfusion salvages ischemic myocardium from infarction, and that infarction spreads in a wave front during ongoing ischemia but leaves salvageable myocardium up 2-3 hours. Then, Murry et al. in 1986 came up with the ingenious idea to perform short cycles of ischemia-reperfusion before a prolonged ischemic insult and found that this maneuver attenuated myocardial infarct size (221), thus establishing the prime cardioprotective paradigm of IPC. Further studies showed that IPC has two different temporal forms: an acute form which confers immediate protection but vanishes after an interval of about 2 hours between the preconditioning stimulus and the event that need protection, and a delayed form which reappears after 24-48 hours, lasts longer but is less protective (222). The acute form of IPC relies on the recruitment of acutely available signaling modules, whereas the delayed form involved increase expression of protective proteins in response to an acute signal (223). IPC has been translated to human beings with ischemic heart disease but because of its nature it can not be used un patients with AMI, and it can be used only in situations as percutaneous coronary interventions and coronary artery bypass grafting (220, 224).

Protection of coronary vascular function by IPC is uncertain as there are results indicating that this procedure preserves coronary vasomotion, whereas other results do not (see Reference 14).

IPOC: Modification of reperfusion can attenuate reperfusion injury and thus reduce infarct size. This procedure refers to intermittent reperfusion of the acute ischemic myocardium, which has been reported to prevent myocardial reperfusion injury and reduce myocardial infarction size by 40%–50% (122). IPOC was established by Z. Q. Zhao et al. (225) who experimentally observed that 3 cycles of 30 sec reperfusion/30 sec reoclusion at the immediate onset of reperfusion after 60 min of coronary occlusion reduced infarct size to a similar amount that with IPC. This seminal study was followed by others. IPOC is limited to the early minutes of reperfusion, and it has been successfully translated to human beings with ischemic heart disease, and can be used in patients undergoing interventional reperfusion of AMI (224). Staat et al. (226) first applied IPOC to the clinical setting of percutaneous coronary artery: immediately after direct stenting, coronary blood reflow was allowed for 60 seconds, following which the angioplasty balloon was inflated upstream of the stent for 60 seconds to occlud coronary blood flow, and this cycle was repeated 4 times in total. The results of this study confirmed the existence of lethal myocardial reperfusion injury in humans (227), but a number of clinical studies have subsequently confirmed the beneficial effects of IPOC, although not all studies have had positive results. Protector effects of IPOC on coronary vasculature functionary unclear (see Reference 14).

RIPC: IPOC requires an invasive therapeutic intervention applied directly to the heart. However, the heart can be protected against acute ischemia-reperfusion injury from a distance, by applying one or more cycles of brief, nonlethal ischemia and reperfusion to another organ or tissue, a phenomenon that has been termed remote ischemic conditioning (RIPC) (228). In the clinical setting, RIPC has been achieved noninvasively by simply inflating and deflating a blood pressure cuff placed on the upper arm to induce three 5-minute cycles of ischemia and reperfusion (229). This therapeutic approach has been reported to be beneficial in patients undergoing cardiac surgery and in patients undergoing elective percutaneous coronary interventions. More recently, Botker et al. (230) demonstrated that RIPC applied by a paramedic to patients with ST segment elevated myocardial infarction in transit to the percutaneous coronary interventions center improved myocardial salvage compared with control patients. A recent review and meta-analysis of clinical studies shows that RIPC could be an effective method for reducing myocardial ischemia-reperfusion injury (231).

These conditioning procedures exert significant cardioprotection, but they may be injurious per se, and a better understanding of the signal transduction under laying the conditioning phenomena may help to make more beneficial the application of these techniques. A review about signaling molecules and mechanisms in conditioning (physical and chemical triggers, intracellular signal transduction) can be found in References 220 and 232. Cardioprotective signal transduction appears as a highly concerted spatiotemporal program, and the general consensus is that mitochondria are the most important effector of protection of conditioning, where most of signaling pathways may converge (220, 232). Although the translation of IPC and RIPC protocols to patients with acute myocardial infarction has been fairly successful, the pharmacological recruitment of cardioprotective signaling has been largely disappointing to date.

4.2. Pharmacological strategies

Several studies to prevent reperfusion injury by means of pharmacological agents have been conducted. Factors of importance are the timing of drug administration, animal species used, degree of collateral flow and the duration of ischemia. A variety of pharmacological compounds have been investigated in different experimental models of myocardial ischemia and reperfusion.

Considering the factors that may contribute to the deleterious effects of myocardial ischemia-reperfusion, pharmacological strategies tested are summarized as follows:

A) Against oxidative stress. Within a few minutes of myocardial reperfusion, a burst of oxidative stress (233) is produced by a variety of sources. This oxidative stress mediates myocardial injury and cardiomyocyte death through a number of different mechanisms. Based on these

observations, antioxidant therapy was considered to be an appropriate option to prevent such injury. However, both experimental and clinical studies have reported mixed results with the administration of antioxidant therapy at the onset of myocardial reperfusion (234). However, the discovery of mitochondrial-specific antioxidants may be more effective (235).

B) Against intracellular Ca2+ overload. Ca2+ overload begins during acute myocardial ischemia and is exacerbated during myocardial reperfusion, and this overload induces the opening of the mitochondrial permeability transition pore. Experimental studies have shown that pharmacologic antagonists of the sarcolemmal Ca2+ channel (236) or the mitochondrial Ca2+ uniporter (237) administered at the onset of myocardial reperfusion, reduces infarct size by up to 50%. However, not all experimental studies using this therapeutic strategy have been positive. Clinical studies of Ca2+ channel blockers administered at the onset of myocardial reperfusion have not shown beneficial results (238). The identification of the mitochondrial Ca2+ uniporter may result in the discovery of a new class of specific inhibitors for targeting lethal myocardial reperfusion injury.

C) To slow the restoration of physiological pH. During acute myocardial ischemia the intracellular pH decreases to <7. 0, where as at reperfusion, physiological pH is rapidly restored by the washout of lactate and the activation of the Na+-H+ exchanger, as well as the Na+-HCO– symporter. This pH shift contributes to the cardiomyocyte death of lethal myocardial reperfusion injury by permitting mitochondrial permeability transition pore opening and cardiomyocyte rigor hypercontracture in the first few minutes of reperfusion. Reperfusion of ischemic animal hearts with an acidic buffer can reduce infarct size (239). Therefore, a potential treatment strategy for preventing lethal myocardial reperfusion injury would be to slow the normalization of physiologic pH at the time of myocardial reperfusion or by slowing the process of myocardial reperfusion, as in the case of ischemic postconditioning (122).

D) The mitochondrial permeability transition pore (MPTP) as a target for cardioprotection. The MPTP is a nonselective channel of the inner mitochondrial membrane, the opening of which results in mitochondrial membrane depolarization and uncoupling of oxidative phosphorylation, leading to ATP depletion and cell death. As such, preventing MPTP opening at the time of reperfusion by administering MPTP inhibitors (e. g., cyclosporin A) at the onset of myocardial reperfusion has been reported in experimental studies to reduce infarct size by 40–50% in animal myocardial infarction models (240). Thus, MPTP may be an important therapeutic target for preventing lethal myocardial reperfusion injury (122).

E) Against inflammatory response. It is unclear whether the inflammatory response that accompanies an AMI contributes to the pathogenesis of lethal myocardial reperfusion injury or whether it is a reaction to the acute myocardial injury. Although experimental studies have reported significant reduction of infarct size by inhibiting the inflammatory process at the time of myocardial reperfusion and the inhibition of complement activation, corresponding clinical studies using this therapeutic approach have been largely negative (122).

F) Against late myocardial reperfusion injury. Some of the described stimulators of myocardial reperfusion injury all appear to operate in the first few minutes of myocardial reperfusion, providing a narrow window for reducing infarct size in patients. However, several other important processes such as apoptosis and inflammation, which are also initiated during ischemia and continue over several hours into reperfusion, may contribute to the development of lethal myocardial reperfusion injury. These contributing pathways provide a potential second therapeutic window for reducing infarct size. However, this is a controversial area of research, and some experimental studies have failed to demonstrate an increase in infarct size with reperfusion time. Several experimental studies have reported that administering cardioprotective agents such as erythropoietin (anti-apoptotic), PI3K-γ/δ inhibitors (anti-inflammatory), and intracoronary aqueous oxygen, from 30 minutes to 24 hours into myocardial reperfusion may still limit acute myocardial infarct size at 72 hours. This may provide an additional therapeutic window to target late into the reperfusion phase (122).

G) Antagonists for endothelin ETA and ETB receptors. As ET-1 may have a relevant role in pathophysiology of myocardial ischemia-reperfusion, this has stimulated the possible beneficial effects of endothelin-receptor-blockade to protect the heart against reperfusion injury. However, this research has yielded unclear results. Respect to this issue, it could be of interest to take in mind the probable interaction between NO and ET-1.

Efficacy of the non-selective endothelin antagonist l-753037 was examined in a model of canine coronary artery occlusion and reperfusion to assess whether blockade of both ETA and ETB receptors would enhance or reduce myocardial ischemic injury. The results suggest that this non-selective endothelin antagonist provides significant myocardial protection primarily by improving regional myocardial flow distribution following reperfusion, and demonstrated no detrimental effects associated with blockade of the ETB receptor (241).

Endothelin ETA and ETB receptor antagonists could be cardioprotective during myocardial ischemia and reperfusion through a NO-dependent mechanism. To investigate whether the ETA and ETB receptor antagonist, bosentan, is cardioprotective in atherosclerotic mice, buffer-perfused hearts from apolipoprotein E/LDL receptor double knockout and wild-type mice were subjected to global ischemia and reperfusion. Following reperfusion, the recovery of left ventricular function was equally impaired in wild-type and double knockout mice given vehicle. The ETA/ETB receptor antagonist bosentan improved recoveries in wild-type and in double knockout

mice. Similar effects were observed for the recovery of left ventricular end-diastolic pressure, developed pressure and dP/dt. Bosentan improved the recovery of coronary flow in both double knockout and wild-type mice. Recovery of coronary flow was significantly higher in the double knockout mice given bosentan than in the wild-type group. ET-1 impaired recovery of coronary flow in both wild-type and double knockout mice though this effect was more pronounced in the double knockout mice. Coronary outflow of NO during reperfusion was enhanced in both double knockout and wild-type mice following bosentan administration. Therefore, the ETA/ETB receptor antagonist bosentan may protect the atherosclerotic mouse heart from ischemia-reperfusion injury. The observation that endothelin receptor blockade and stimulation have a greater effect on coronary flow in atherosclerotic hearts indicates an increased activation of the endothelin system in atherosclerotic coronary arteries (242).

In our laboratory we have compared the effects of antagonists for endothelin ETA and ETB receptors on the action of ischemia-reperfusion on endothelial and myocardial function. The study we carried out in anesthetized goats, in which 30 min of partial or total occlusion followed by 60 min of reperfusion of the left circumflex coronary artery was induced in animals treated with intracoronary administration of saline (vehicle), BQ-123 (endothelin ETA receptors antagonist) or BQ-788 (endothelin ETB receptors antagonist). During reperfusion after partial occlusion, coronary vascular conductance and left ventricle contractility were decreased after saline or BQ-788, and they normalized after BQ-123. In these three groups of animals, the coronary effects of Ach and sodium nitroprusside during reperfusion were as under control. During reperfusion after total occlusion, coronary vascular conductance and left ventricle function were decreased after saline, and they normalized after BQ-123 or BQ-788 treatment. In these three groups of animals, the coronary effects of Ach but not those of sodium nitroprusside during reperfusion were decreased after saline, and they reversed after BQ-123 or BQ-788 treatment. Therefore, selective antagonists of endothelin ETB and ETA receptors may produce similar protection of coronary vasculature and myocardium against reperfusion after severe ischemia. Selective antagonists of endothelin ETB receptors, contrarily to those of endothelin ETA receptors may be ineffective to protect coronary vasculature and myocardium against reperfusion after mild ischemia. Probably it may be more beneficial the use of antagonists for both subtypes of endothelin receptors (243). In addition of antagonists for ETA receptors, the use of antagonists for ETB receptors is supported by studies performed in pig coronary arteries (202) in which it seems to be that ischemia-reperfusion increases the coronary vasoconstriction in response to ET-1 probably due to impairment of endothelin ETB receptor-induced release of NO and prostacyclin, to augmentation of the contractile response to activation of endothelin ETB receptors, and to increased release of vasoconstrictor prostanoids.

Left ventricular hemodynamic variables were measured in the Langendorff-perfused model after 40- and 20-minute regional or global ischemia, followed by 30-minute reperfusion. Wild-type and ETB-deficient rats were compared. Left ventricular dysfunction was more prominent in ETB-deficient rats, particularly after regional ischemia. Infarct size was smaller in wild-type than ETB-deficient rats after 40 minutes of regional ischemia-reperfusion. Although the recovery of left ventricular function was poorer after 40-minute ischemia-reperfusion, end-diastolic pressure in ETB-deficient rats was higher after 20 than after 40 minutes of regional ischemia-reperfusion. It is concluded that ETB receptors exert cytoprotective effects in the rat heart, mainly after regional ischemia-reperfusion. Longer periods of ischemia suppress the recovery of left ventricular function after reperfusion, but the role of ETB receptors may be more important during the early phases (244).

In rabbits subjected to 1 h of coronary artery occlusion followed by 3 h of reperfusion, left ventricle unloading was initiated 15 min prior to reperfusion and was maintained during reperfusion. Animals were treated with the ETA receptor antagonist BQ123. In parallel, isolated rabbit cardiomyocytes subjected to simulated ischemia-reperfusion with or without ET-1 or BQ123, intracellular calcium and cell death were assessed with flow cytometry. From the results of this study, the authors suggest that components of reperfusion injury involve ET-1 release which stimulates calcium overload and apoptosis. Intravenous ETA receptor blockade prior to reperfusion may be a protective adjunct to reperfusion therapy in acute myocardial infarction patients. Thus, endogenous ET-1 released during acute myocardial infarction might mediate ischemia-reperfusion injury by stimulating increased intracellular calcium concentration, and apoptosis (245).

In patients with type 2 diabetes and coronary artery disease it has been observed that both selective ETA and dual ETA/ETB receptor antagonists improve endothelium-dependent vasodilatation. ETB receptor blockade increases basal blood flow but does not additionally improve endothelium-dependent vasodilatation (246). Safety and feasibility of selective ETA receptor blockade in ST-elevation acute coronary syndrome (STE-ACS) within a larger randomized trial was assessed in patients. Patients with posterior-wall STE-ACS were randomly assigned to receive intravenous BQ-123 or placebo over 60 min, starting immediately prior to primary percutaneous coronary intervention. Twenty-four hour Holter recordings were performed during hospitalization for STE-ACS and after 6–8 weeks. The predefined primary endpoint was the documentation of ventricular tachycardia and/or late potentials at follow-up. Based on the analysis of long-term ECG data, short-term administration of BQ-123 after AMI was safe (247).

The studies shown before support the idea that ET-1 could play a relevant role in the adverse effects of ischemia-reperfusion on the myocardium, including coronary vasculature. These adverse effects of ET-1 may

be mediated by both endothelin ETA and ETB receptors; endothelin ETB receptors situated in the endothelium may reduce their function probably due to endothelium damage, whereas those situated in smooth muscle cells of coronary arteries acquires a high role and thus contributing, together with endothelin ETA receptors, to the adverse coronary effects of ET-1. Thus, the treatment with blockers of both endothelin ETA and ETB receptors may protect the myocardium and coronary vasculature against the adverse effects of ischemia-reperfusion, and in consequence it may reduce reperfusion injury.

H) Urocortin. This substance is a 40 amino-acid peptide which has a high degree of structural homology with the peptide corticotrophin-releasing factor (CRF), and has marked cardiovascular effects. In the heart, exogenous urocortin produces coronary vasodilation (248). Also, it has been demonstrated that urocortin production may be increased in cardiac cells exposed to ischemia and that exogenous urocortin may protect myocardium during coronary ischemia (249), and that urocortin reduces the infarct area in the ischemic and reperfused rat heart (250). Several mechanisms may be involved in this protective effect of urocortin in the myocardial cells (251). To explore the action of urocortin on the adverse effects of ischemia-reperfusion on coronary vasculature, hearts from rats were perfused at constant flow and then exposed to 15 mins ischemia followed by 15 mins reperfusion. In one series of experiments, we found that the coronary relaxation to urocortin was reduced after ischemia-reperfusion. Treatment with a low threshold concentration of urocortin, administered before ischemia and during reperfusion, improved the coronary relaxation to Ach after ischemia-reperfusion. Therefore, this condition impairs the coronary vasodilation to urocortin and produces endothelial dysfunction, and this endothelial dysfunction may be improved by urocortin (252).

In other series of experiments (253), we observed that the urocortin-induced improvement of the coronary relaxation to Ach after ischemia-reperfusion was not modified by treatment with tetraethylammonium, blocker of Ca2+ dependent-potassium channels; glibenclamide, blocker of K (ATP) channels; L-NAME, blocker of NO synthesis; or meclofenamate, blocker of cyclooxygenase, but it was abolished by chelerythrine, blocker of protein kinase C Thus, it is suggested that urocortin may protect coronary endothelial function during ischemia-reperfusion by activation of protein kinase C. Therefore, urocortin may act as an endogenous protective factor of the heart during ischemia. These protective effects of urocortin may act on the endothelial function (252) as well as on the myocardial function (249, 250) during ischemia-reperfusion. Urocortin may activate ATP-sensitive and Ca2+-sensitive potassium channels in cardiac myocytes and in vascular smooth muscle cells, respectively. However, we found that neither glibenclamide nor TEA modified the urocortin-induced improvement of the coronary endothelial function, suggesting that these channels do not seem to be involved in the protective effect of urocortin on endothelial cells during ischemia-reperfusion. Brar et al also suggest that the protective effect of urocortin on the endothelial cells during ischemia-reperfusion may be mediated by activation of protein kinase C, as an inhibitor of this enzyme, chelerythrine, abolished the improvement of endothelial function by urocortin (249). Gordon et al. (254) also found that the protection by urocortin against ischemia-reperfusion in adult rat cardiomyocytes was attenuated by chelerythrine. Also, our results partly agree with those of Lawrence et al. (255), who showed involvement of protein kinase C epsilon in the prevention of mitochondrial damage during ischemia-reperfusion. However, in the studies of Gordon et al. (254) and Lawrence et al. (255), KATP channels were also involved, a phenomenon that was not observed in our study. It may be supposed that urocortin action may share some mechanisms, but not others, in endothelial and myocardial cells. In conclusion, our results suggest that relatively low concentrations of urocortin may protect endothelial function in the coronary circulation against deleterious effects of ischemia-reperfusion through activation of protein kinase C. Urocortin might act as an endogenous protective factor in the heart during coronary ischemia-reperfusion, and identification of its protective mechanisms on endothelial function may help to prevent the coronary vascular dysregulation that occurs after heart ischemia (252, 253). With respect to this, it is remarkable that a recent study in humans (256) provided the first evidence that human urocortin prevents the development of atherosclerosis by suppressing endothelial cell inflammatory response and proliferation, macrophage foam cell formation, and vascular smooth muscle cell migration and proliferation. Thus, human urocortin might serve as a novel therapeutic agent for atherosclerotic cardiovascular diseases (256).

5. CONCLUSIONS

Acute coronary syndromes (e. g., acute myocardial infarction (AMI)) are the most lethal of cardiovascular diseases, but the mortality rates of these syndromes are going down in developed countries as a result of better prevention and treatment. The most effective treatment of AMI is timely (early) reperfusion of the myocardial ischemic zone. However, not all it is favorable with myocardial reperfusion as this procedure may also damage myocardium, which is known as “reperfusion injury”.

The present Review pays particular attention to the coronary circulation and to the role of NO and ET-1 in the regulation of this circulation under normal conditions and after ischemia-reperfusion. Also, it is considered therapeutical strategies for ischemia-reperfusion injury, with special mention to antagonists for endothelin receptors. The coronary circulation plays a crucial role in pathophysiology of ischemia-reperfusion and reperfusion injury, after all it is the cause and victim of this condition. Ischemia-reperfusion damages not only cardiomyocytes, but also coronary vasculature. The damage to this vasculature may vary from functional impairment of

endothelium to severe structural alterations of the vasculature, and finally to vascular thrombosis and the no-reflow phenomenon, which may underly reperfusion injury. The endothelium plays a relevant role in the regulation of the coronary circulation, and it could exist a functional interaction between NO and ET-1 in the coronary artery wall, with predominance of NO over ET-1 and also with a relevant role of NO in modulation of coronary effects of ET-1under normal conditions. The endothelium seems to be highly sensitive to ischemia, and after ischemia-reperfusion the endothelial function and the interaction between NO and ET-1are altered, and now ET-1 predominates over NO and the modulator role of NO in the effects of ET-1 is decreased. These features lead to decreased endothelium-dependent coronary vasodilatation, particularly that mediated by NO, and also to increased coronary vasoconstrictor response to ET-1. After ischemia-reperfusion, the production of ET-1 seems to be augmented, and the equilibrium between endothelin receptors may be altered with an increased role of the smooth muscle cells ETA and ETB receptors in the augmented coronary vasoconstriction to ET-1. It is probable that ischemia-reperfusion by damaging the endothelium it decreases the NO-mediated vasodilation of coronary vessels, and at the same time it augments the production of ET-1 and its coronary vasoconstrictors effects. This damage of the endothelium and the augmented presence of ET-1 and of its coronary effects, would contribute to produce vascular dysfunction, vascular damage, vascular thrombosis and the non-reflow phenomenon, thus contributing to reperfusion injury. From these considerations, it can be suggested that ET-1 plays a significant role in pathophysiology of coronary ischemia-reperfusion and of reperfusion injury, and therefore it is reasonable to expect that the use of antagonists for endothelin ETA and ETB receptors could be beneficial for protecting the heart against this condition.

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