70

Cardiovascular Regulation: Basic Considerations

Giuseppe Mancia, Thomas F. Lüscher, John T. Shepherd, George Noll, and Guido M. Grassi

Key Points

- Homeostatic control of the cardiovascular system depends on a variety of metabolic, humoral, and neuroadrenergic influences. These factors physiologically interact with each other and participate, together with endothelial factors, in the regulation of cardiac as well as vascular function.
- Both endothelial and sympathetic functions undergo a variety of physiologic adjustments, participating in the cardiovascular responses to postural changes, to environmental temperature modifications, to stress, and to physical exercise. They also participate in the cardiovascular modifications typical of the aging process.
- In cardiovascular and metabolic diseases, sympathetic and endothelial alterations represent the key factors for the pathophysiology of the clinical conditions, its complications, and prognosis. They also represent the target for nonpharmacologic and pharmacologic interventions.

Cardiovascular homeostasis represents the mechanism(s) through which organ perfusion, metabolic balance, and thermoregulation are modulated to meet the body's requirements. This demands complex interplays among local, humoral, and neural factors to modify cardiac and vascular performance according to the changing requirements of daily life. In diseases of the cardiovascular system (as well as of other organs, such as the kidney and the liver), these regulatory mechanisms are disturbed with consequent abnormalities in circulatory control.

Cardiovascular Regulation in Physiologic Conditions

Local Factors

Endothelial cells play a leading local homeostatic role by secreting vascular relaxing and contracting substances that act locally to modify the tone of the underlying smooth muscle.[1,2] The major relaxing factor is nitric oxide (NO); others are prostaglandin I_2, (also termed prostacyclin), endothelium-derived hyperpolarizing factor,[2] and C-type natriuretic peptide (CNP). The major contracting factor is endothelin (ET)-1; others are angiotensin II (Ang II) and vasoconstrictor metabolites of arachidonic acid. These locally synthesized substances also modulate the response of the underlying vascular smooth muscle to hormones, neurotransmitters, and platelet products (Fig. 70.1). In addition, the endothelium releases tissue plasminogen activator (tPA) involved in the modulation of the fibrinolytic process.

Endothelium-Derived Relaxing Factors

In the endothelial cells, the constitutive enzyme nitric oxide synthase (eNOS) converts L-arginine to L-citrulline with a release of NO. A cofactor, tetrahydrobiopterin, is required for activation of nitric oxide synthase (NOS).[3] Nitric oxide activates soluble guanylate cyclase in the underlying smooth muscle, and the resultant increase in cyclic guanosine monophosphate causes its relaxation. The NO is inactivated within a few seconds by superoxide anions.

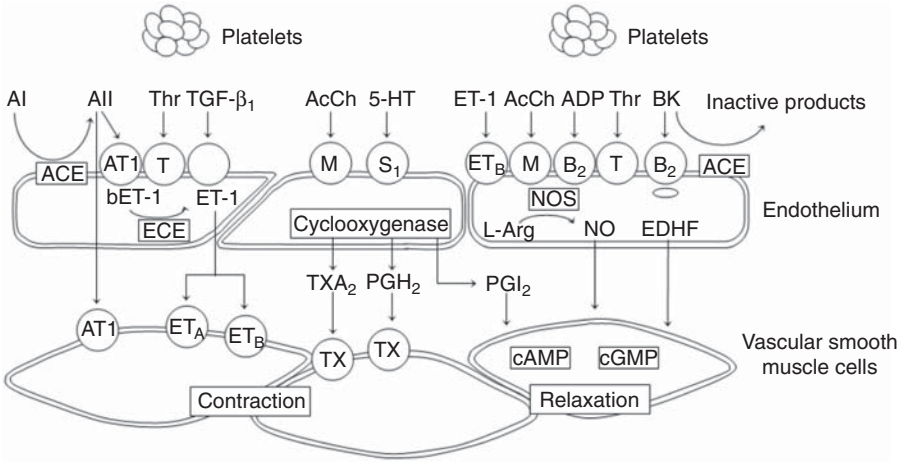

FIGURE 70.1. Endothelium-derived vasoactive substances. Nitric oxide (NO) is released from endothelial cells in response to shear stress and to activation of a variety of receptors. It exerts vasodilating and antiproliferative effects on smooth muscle cells and inhibits thrombocyte-aggregation and leukocyte-adhesion. Endothelin-1 (ET-1) exerts its major vascular effects of vasoconstriction and cell proliferation through activation of specific ET_A receptors on vascular smooth muscle cells. In contrast, endothelial ET_B receptors mediate vasodilation via release of NO and prostacyclin. Additionally, ET_B receptors in the lung were shown to be a major pathway for the clearance of ET-1 from plasma. ACE, angiotensin-converting enzyme; Ach, acetylcholine; AII, angiotensin II; AT1, angiotensin 1 receptor; BK, bradykinin; COX, cyclooxygenase; ECE, endothelin-converting enzyme; EDHF, endothelium-derived hyperpolarizing factor; ET_A and ET_B, endothelin A and B receptor; ET-1, endothelin-1; PGH_2, prostaglandin H_2; PGI_2, prostacyclin; S, serotoninergic receptor; Thr, thrombin; T, thromboxane receptor; TXA_2, thromboxane; 5–HT, 5–hydroxytryptamine (serotonin).

Nitric oxide not only is a potent vasodilator but also inhibits platelet aggregation and leukocyte adhesion to endothelial cells and suppresses the proliferation and migration of vascular smooth muscle cells. The expression of eNOS is regulated by the action of shear stress on the endothelial cells and by the cyclic circumferential stretch of the blood vessels.[4] Unidirectional shear stress increases eNOS messenger RNA (mRNA) expression via a transcriptional mechanism, whereas oscillatory shear stress and cyclic stretch do this through posttranscriptional regulatory events.[5] The earliest mechanochemical signal transduction is activation of specific G proteins in the endothelium within 1 second of flow-induced signaling.[6] The biologic effects of NO are determined by the amount released and its inactivation by superoxide anions (O_2^-). The endothelial cells are a source of superoxide, and NOS can produce superoxide.[7] Tetrahydrobiopterin determines the balance of O_2^- and NO production from eNOS after prolonged stretch of human aortic endothelial cells,[8] its deficiency favoring a coronary circulatory dysfunction.[9]

Nitric oxide synthase also may catalyze formation of hydrogen peroxide (H_2O_2). This is favored by low endogenous concentrations of L-arginine, tetrahydrobiopterin, or both. Although H_2O_2 is a potent vasodilator, prolonged increased concentrations of H_2O_2 may be harmful to endothelial and smooth muscle cells, leading to a shift in the balance between the production of protective NO and deleterious O_2^-.[10,11] In addition, O_2^- from the adventitia of the blood vessel can inactivate NO.[12] Oxygen-derived free radicals have been implicated in the pathogenesis of atherosclerosis and vascular restenosis.[13] Hypercholesterolemia, diabetes, and ischemia followed by reperfusion are also associated with increased vascular O_2^- production. Native low-density lipoproteins and Ang II have been reported to stimulate O_2^- production from endothelial and vascular smooth muscle cells. Moreover, the proliferative response of smooth muscle cells to platelet-derived growth factor is mediated by H_2O_2. Pulsatile stretch applied to human coronary artery smooth muscle cells causes their proliferation, which is associated with increased oxidative stress. This increase promotes DNA synthesis in these muscles.[14]

Nitric oxide forms complexes with various biomolecular carriers such as nitrosothiol (RS-NO) that retain biologic activity. Studies on the forearm resistance vessels of normal human beings indicate that RS-NO contributes to vascular smooth muscle relaxation.[15] Basal and flow-induced release of NO from vascular endothelium can be mediated via local cholinergic mechanisms. The flow may cause acetylcholine release from certain endothelial cells, which stimulates NO release from these cells or from neighboring endothelial cells.[16]

There are several receptors in the endothelial cells that, if activated, cause a release of NO. Some of the norepinephrine released when the sympathetic nerves are activated stimulates α_2-adrenoceptors on the endothelial cells. The resultant release of NO attenuates the vasoconstriction.[17] Other agonists include bradykinin, histamine, and substances released from platelets [adenosine triphosphate, 5-hydroxytryptamine (serotonin), and thromboxane A_2]. Nitric oxide, but not prostacyclin, is essential for flow-mediated dilatation of large human arteries.[18]

There are two other important roles for NO: first, to maintain a balance in the kidney between oxygen consumption and sodium reabsorption[19]; and second, NO released

from the vascular endothelium plays an important role in the regulation of tissue mitochondrial respiration in skeletal muscle[20] and in the regulation of cardiac contractile function.[21] Another important finding is that in healthy conscious adults, the pulmonary vascular resistance is maintained in part through the continuous local production of NO.[22] Pulsatile stretch in coronary arteries also can release endothelium-derived hyperpolarizing factor.[23] C-type natriuretic peptide (CNP) is produced in endothelial cells and has been proposed to mediate vascular relaxation by causing endothelium-dependent hyperpolarization. To study this in porcine coronary arteries, the endothelium-dependent relaxation and hyperpolarization of CNP and bradykinin were compared. In contrast to bradykinin, CNP induced endothelium-independent and weaker relaxation and hyperpolarization of coronary artery vascular smooth muscle, suggesting that it is an unlikely mediator of endothelium-dependent hyperpolarization of porcine coronary arteries.[24]

Endothelium-Derived Contracting Factors

Endothelin-1, which is produced by endothelial cells of blood vessels, is a potent vasoconstrictor peptide. It is a member of a family of 21-amino-acid peptides consisting of three isosforms: ET-1, ET-2, and ET-3. ET-1 is the only one produced by endothelial cells. It has additional actions including interactions with the sympathetic nervous and renin-angiotensin system, potentiation of responses to other constrictor agents, and stimulation of mitogenesis of vascular smooth cells and cardiomyocytes.[25,26] It also appears to have major effects on cardiac, renal, and cerebral function.[27]

The mechanism of ET-mediated vasoconstriction involves binding to specific receptors on vascular smooth muscle and direct activation of voltage-operated calcium channels in vascular smooth muscle membrane. Two distinct complementary DNAs (cDNAs) of ET receptors have been identified. The ET_A receptor is expressed in vascular smooth muscle cells, whereas the ET_B receptor has been localized to the endothelial and smooth muscle cells. The ET_B receptor may have a dual vasoconstrictive and vasodilatory effect.[28] An $ET_{A/B}$ receptor antagonist decreases peripheral vascular resistance and, to a lesser extent, arterial blood pressure. It increases circulating ET concentrations and blocks forearm vasoconstriction to exogenous ET-1. These results suggest that endogenous generation of ET-1 plays an important physiologic role in the maintenance of peripheral vascular tone and blood pressure in humans.[29] While in vivo selective ET_A receptor antagonism causes forearm vasodilatation in resistance vessels due mostly to increased NO generation, stimulation of ET_A receptors triggers acute vasoconstriction of large conduit arteries.[30] The ET_B receptor antagonism causes local vasoconstriction, indicating that these receptors in blood vessels respond to ET-1 predominantly by causing vasodilatation.[31] Acute elevations in plasma ET-1 concentrations in the coronary artery within a pathophysiologic range do not impair blood flow to normal or collateral vessel-dependent myocardium. This is because increased prostacyclin production in response counteracts the vasoconstrictor properties of ET-1.[32]

There are complex interactions between endothelium-derived substances, including ET-1 and NO. ET-1 induces the formation of NO, which is believed to mediate its vasodepressor action. Furthermore, endothelium-derived NO inhibits the synthesis and may also counteract the vasoconstrictor and vasopressor actions of ET-1. In addition, both NO and ET-1 have been implicated in the regulation of blood and plasma volume and albumin extravasation in various vascular beds.[33] Other endothelium-derived contracting factors that are less important than ET-1 are angiotensin II, thromboxane A_2, prostaglandin H_2, and oxygen-derived free radicals (Fig. 70.1).[25,34]

Autonomic Nerves, Neurotransmitters, and Vascular Receptors

In 1946, Von Euler demonstrated that norepinephrine was released on activation of the sympathetic nerves. Adenosine triphosphate and neuropeptide Y are cotransmitters in these nerves. In the smooth muscle of the systemic vessels, norepinephrine excites α_1-and α_2-adrenoceptors, adenosine triphosphate $P_{2\chi}$ purinoceptors, and neuropeptide Y_1 receptors to cause vasoconstriction.[35] In contrast, in the coronary arteries, the simultaneous activation of β-adrenoceptors and $P_{2\chi}$ receptors results in their dilation. Neuropeptide Y also enhances the activity of norepinephrine and adenosine triphosphate on their receptors.[36] α_2-Adrenoceptors and neuropeptide Y_2 receptors also are present on the sympathetic nerve varicosities. If these are activated, there is a decrease in the output of norepinephrine and adenosine triphosphate.

Interactions between sympathetic and parasympathetic influences at cardiac level have traditionally been defined in terms of their classic neurotransmitters: norepinephrine and acetylcholine. It is known that neuropeptides, which are released from the sympathetic nerves during their activation, have powerful and long-lasting inhibitory actions on vagal transmission in the heart.[37] Long-term sympathectomy causes a decrease in eNOS, 5-hydroxytryptamine, and substance P, and an increase in ET-1 immunoreactivity in the thoracic aortic endothelium of the rat.[38] In addition, nerves that function by releasing NO have been discovered (nitroxidergic or nitrergic nerves) and, in the vessels from different species so far examined, are distributed to the cerebral, femoral, mesenteric, penile, renal, and retinal arteries.[39,40]

A local sympathetic venoarteriolar axon reflex has been identified that contributes to the maintenance of arterial blood pressure in humans on assumption of the upright position.[41] In secondary Raynaud's phenomenon the presence of an impaired venoarteriolar reflex has a high prevalence and indicates the occurrence of a local vasomotor dysfunction.[42]

Cardiovascular Reflexes

Changes in sympathetic outflow are governed by arterial baroreceptors and chemoreceptors, cardiopulmonary mechanoreceptors, and receptors located in skeletal muscles that are activated by muscular contraction (Fig. 70.2). Changes in sympathetic outflow also can occur due to primary changes in the activity of particular centers in the brain. To meet the various stresses to which the human body is exposed, the sympathetic outflow occurs in a differentiated pattern. Thus, in response to reflex or central stimuli, the efferent sympathetic activity varies among the different organs and tissues,

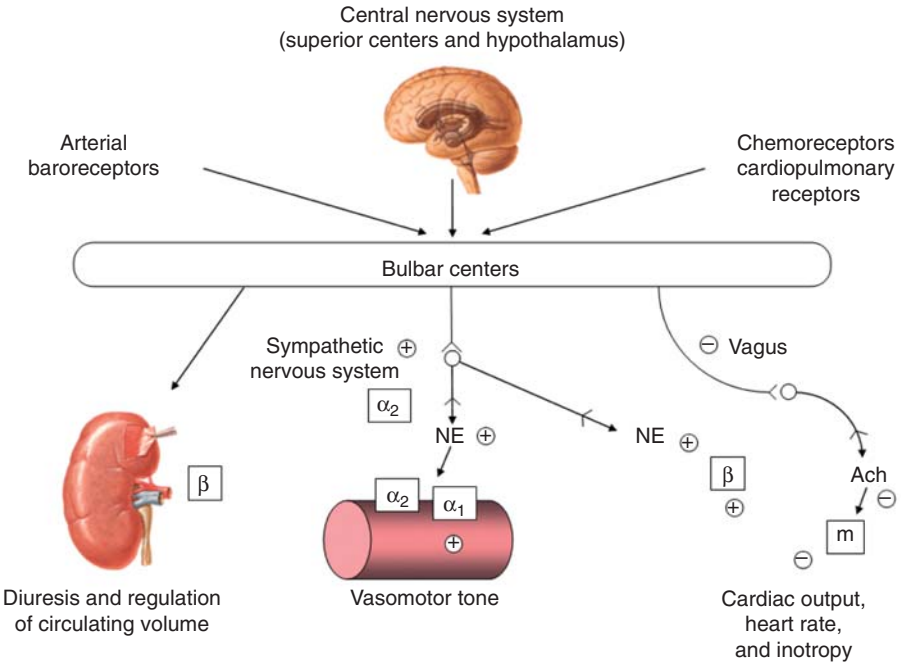

FIGURE 70.2. Autonomic control of the cardiovascular system, with particular emphasis on modulation of (1) kidney function, (2) vasomotor function, and (3) cardiac function. The symbol + refers to the excitatory influences, whereas the symbol—refers to the inhibitory ones. Ach, acetylcholine; NE, norepinephrine; M, muscarinic receptors; β, β-adrenoreceptors; α_1 and α_2, α_1 and α_2 adrenoreceptors.

and in the same organ or tissue can vary between resistance and capacitance vessels. In some instances, sympathetic activity may increase in some organs and decrease in others. For example, in essential hypertension, obesity, and congestive heart failure (CHF), sympathetic nerve activity is increased to muscle, but not to skin, vessels.[43]

Arterial blood pressure and heart rate change not only in relation to behavioral and environmental factors but also as a result of cyclic fluctuations unrelated to external stimuli. Blood pressure variability includes rhythmic and nonrhythmic oscillations.[44] Physical training improves the baroreceptor control of the systemic circulation via the sympathetic nervous system, and this effect may be different from the concomitant effect of training on arterial or baroreceptor control of cardiac sympathetic activity.[45] In addition to the importance of local factors at the site of the mechanoreceptors in the carotid sinus and aortic arch in regulation of the autonomic outflow to the heart and circulation, studies in conscious rabbits have shown that NO in the brainstem plays an important role in the rapid central adaptation of baroreflex control of sympathetic nerve activity.[46] Also, an elevated level of Ang II is critical for the inhibitory effect of NO on sympathetic outflow.[47] Similar findings have been recently reported in human beings.[26,48] Mechanoreceptors in the heart and lungs are important in the reflex control of the circulation. In humans, this reflex influence includes vascular resistance, plasma renin activity, and plasma vasopressin levels, indicating a role for these receptors in both blood volume and blood pressure control.[49]

Nitroxidergic (Nitrergic) Nerves

Nitroxidergic nerves have a constitutive neuronal isoform of NOS and cause vasodilatation through the release of NO. Because NO is a labile-free radical, unlike other transmitters, it is not stored in synaptic vesicles and is not released by exocytosis but instead diffuses from the nerve terminals into adjacent cells.[39,40,50]

Interactions Between Neurotransmitters and Endothelial Cells

Are the actions on the vascular system of the neurotransmitters and the endothelium-derived vasoactive substances the sum of their separate effects, or are they modified by interactions between them? This depends on the ability of any neurotransmitter to diffuse through the vascular wall and to affect the specific endothelial receptors.[51] In the coronary arteries, acetylcholine released from the vagal nerves causes their dilatation through the activation of a muscarinic receptor on the endothelial cells, leading to a release of NO.[52] In other studies aimed at examining whether autonomic influences modulate vascular NO-mediated vasodilatation or even directly contribute to production of NO via nitroxidergic fibers, it was found that tonic NO-dependent vasodilatation can be physiologically maintained in unanesthetized, unrestrained rats regardless of autonomic or humoral adrenergic influences.[53]

Humoral Factors

Angiotensin II

The vasoconstrictor peptide Ang II plays an important role in the control of systemic blood pressure. In addition to its direct action on blood vessels, it facilitates sympathetic influences on the cardiovascular system, by acting both at a central and a peripheral neural level, thereby potentiating the vasoconstrictor effects induced by adrenergic stimuli (Fig. 70.3).[54]

It seems that Ang II can stimulate the synthesis of eNOS and hence enhance the production of NO.[26,48] It is suggested

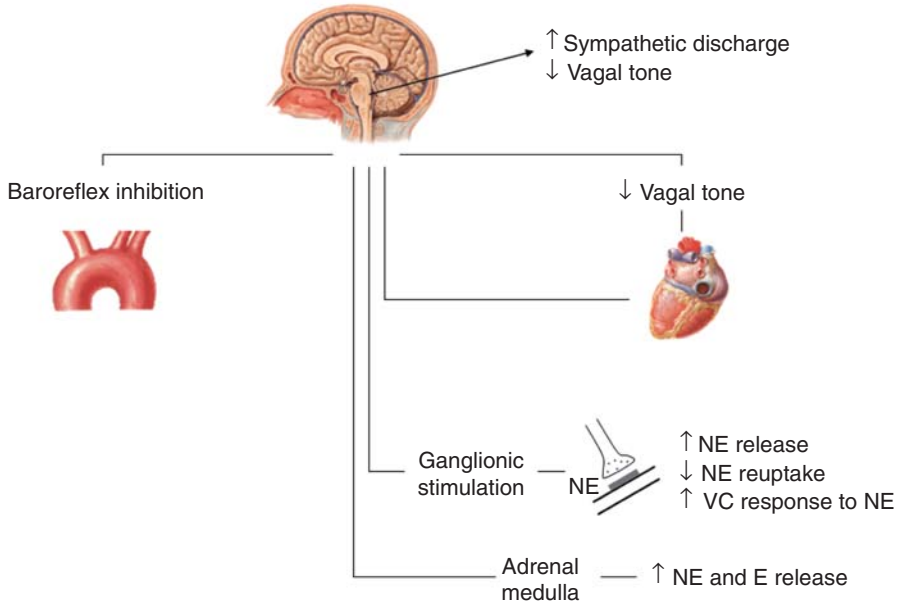

FIGURE 70.3. Central and peripheral sites of interaction between the renin-angiotensin and the sympathetic nervous system. Mechanisms of interaction are also schematically depicted. NE, norepinephrine; E, epinephrine; VC, vasoconstriction.

that in the renal circulation, this Ang II–NO interaction may protect the preglomerular vessels from the constrictor effect of Ang II.[55] In patients with coronary artery disease, angiotensin-converting enzyme (ACE) inhibitors attenuate sympathetic coronary vasoconstriction, not only when the drugs are systemically administered,[56] but also when small doses of the compounds are infused at the level of the coronary circulation (Fig. 70.4).[57] This finding suggests that not only systemic but also the local renin-angiotensin system is important for preserving cardiovascular homeostasis via an interaction with endothelial factors.

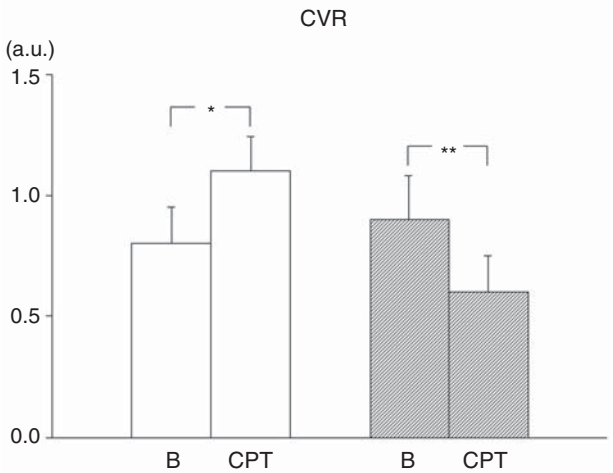

FIGURE 70.4. Effects of cold pressor test (CPT) on coronary vascular resistance (CVR) before (open bars) and after (dashed bars) acute blockade of the coronary renin-angiotensin system (RAAS) via intracoronary infusion of small doses of an ACE inhibitor devoid of systemic effects. Note that while in the control state the maneuver causes a marked coronary vasoconstriction, after acute blockade of RAAS it elicits coronary vasodilation. a.u., arbitrary units (* j < 0.05, ** j < 0.01) refer to the statistical significance between B and CPT.

Estrogens

Both endothelial and vascular smooth muscle cells possess estrogen receptors. Estrogens regulate the transcription of numerous genes, and its cellular actions are mediated through the translation of specific mRNA transcripts and synthesis of proteins. It stimulates the native synthesis of NO in blood vessels, heart, and skeletal muscles.[58,59] This may be achieved via both genomic and nongenomic pathways.[60]

Estrogens enhance the binding activity of the transcription factor Spl, whose function is essential for eNOS transcription. Even modest increases in eNOS expression may display protective effects against cardiovascular disease.[61] Other potential protective mechanisms of estrogen are represented by (1) the suppression of a prostaglandin H synthase-dependent vasoconstriction,[62] and (2) the inhibition of cyclooxygenase-dependent production of oxidative stress.[63] In perimenopausal woman, estrogens supplementation reduces arterial blood pressure and enhances basal NO release in forearm resistance arteries.[64]

Adrenomedullin

Adrenomedullin is a recently identified vasorelaxing and natriuretic peptide. It may exert regulatory effects on cardiac function, because adrenomedullin and its binding sites have been detected in the heart. It enhances cardiac contractility via cyclic adenosine monophosphate–independent mechanisms.[65]

Insulin and Insulin-Like Growth Factor I

There are three peptide hormones in the insulin growth factor family-insulin and insulin-like growth factors (IGFs)-I and -II. Insulin is synthesized and secreted by the pancreas. Although the liver is the main source of circulating IGF-I levels, it is also formed in endothelial and vascular smooth muscle cells. In addition to their metabolic and growth-

promoting actions, these peptides trigger both vasoconstrictor and vasodilator effects on the vascular system.[66,67]

Some studies suggest that endothelium-derived NO mediates the vasodilator actions of insulin and IGF-I,[68] but others have disagreed. We must recognize, however, the heterogeneity of endothelium-mediated responses in different arteries and veins, even within the same species. In isolated porcine coronary arteries, both insulin and IGF-I caused non–endothelium-dependent coronary relaxation, probably through a mechanism involving the activation of potassium channels.[69]

Aging Process

Human aging is associated with a number of complex and diverse changes in the cardiovascular function and structure. These include, for example, a slight increase in left ventricular wall thickness, a slight reduction in diastolic function, a clear-cut decrease in the cardiac content and function of β-adrenergic receptors as well as an impairment in arterial distensibility.[70,71] Findings provide support for the concept that cardiopulmonary and integrative baroreflex control of sympathetic nerve activity during acute hypovolemia is enhanced rather than depressed in healthy older humans.[72] This may help minimize the functional impact of a marked age-related reduction in peripheral vasoconstrictor responsiveness to sympathetic neural stimulation and contribute to the effective regulation of arterial blood pressure in older adults during orthostatic challenge.[71] Aging in humans, however, is associated with an impairment of arterial blood pressure homeostasis. This is reflected by an increased blood pressure variability and a greater decrease in pressure during orthostatic stress compared with younger subjects. This impairment is explained in part by a decreased buffering role of the arterial baroreflex,[49] which, however, appears to selectively affect the modulation of sinus node activity (and thus of heart rate), with no impairment in the baroreceptor control of efferent sympathetic nerve traffic (and thus of blood pressure) being described in patients at advanced ages.[73,74] Several other neurovascular disorders, including a diminished endothelium-dependent vasodilatation and a generalized sympathetic activation (probably dependent not on a baroreflex dysfunction but rather on an age-related increase in body weight with an accompanying insulin resistance state), have been demonstrated in aging humans.[75–77] In aging rats, eNOS activity and NO production are reduced, which could explain the observations in humans.[78,79]

Orthostatic Stress

Gravitational stresses, which are common daily events for humans, result in a reduction in central blood volume due to the displacement of circulating blood to the lower parts of the body. Complex adjustments in the cardiovascular system are required to offset the decrease in cardiac filling pressure. Such changes are necessary to sustain arterial blood pressure at an appropriate level, thus guaranteeing an adequate perfusion of vital organs, especially the brain. These adjustments must compensate for both the initial and the sustained orthostatic stresses. The rapid short-term adaptations are mediated primarily by the cardiovascular reflexes, with humoral agents reinforcing these reflexes during severe and prolonged orthostatic stress.[49]

Pressure receptors (mechanoreceptors) in the heart and great vessels continuously relay information on blood pressure in these areas to the cardiovascular centers in the brain system. A decrease in blood pressure excites the centers with a resultant increase in sympathetic and a decrease in vagal outflow and vice versa.[49] An enhanced sympathetic drive to the heart and blood vessels and a decreased cardiac vagal activity trigger an increase in heart rate and cardiac output and constriction of resistance vessels in skeletal muscle, in the renal and splanchnic bed, and of the venous capacitance vessels in the splanchnic bed. The latter contributes importantly to maintenance of the cardiac filling pressures and, hence, of the stroke volume. The marked sensitivity and rapidity of the reflex responses of the splanchnic capacitance vessels to a very low frequency of sympathetic discharge indicate their importance in regulation of the stroke volume. An important adjunct to the central activation of the vasomotor outflow is the local sympathetic venoarteriolar axon reflex.[41] In addition, if the orthostatic stress is accompanied by contraction of the postural muscles, the decrease in vagal and the increase in sympathetic outflow can be potentiated and sustained by two mechanisms. One is a "central command" related to the motor signals from higher brain centers that stimulates the brainstem cardiovascular centers, and the other is a feedback reflex from the contracting muscles due to activation of their mechanoreceptors and metaboreceptors. The three main humoral factors involved in the maintenance of cardiovascular homeostasis during prolonged orthostatic stress are the renin-angiotensin-aldosterone system, vasopressin, and the atrial natriuretic peptides.[80]

Mental Stress

Mental stress in humans results in arterial hypertension and tachycardia. In the offspring of hypertensive parents, sympathetic activation during mental stress is increased compared with the offspring of normotensive parents.[81] Mental stress also causes a neurogenically mediated vasodilatation in the skeletal muscle. The dilatation is absent after surgical sympathectomy and is blunted after intraarterial administration of atropine. It has been shown that NO plays a key role in the autonomic control of the circulation during stress, with most of the NO release being due to autonomic nerve cholinergic stimulation of the vascular endothelium in the muscles.[82] In line with these findings, recent data provide evidence that acute mental stress, even when of short lasting duration, may exert adverse effects on endothelium by impairing NO-dependent vasodilation.[83]

Thermal Stress

Although maintenance of a constant body temperature results from the interplay between a number of homeostatic mechanisms, the human body undergoes a variety of cardiovascular modifications to counteract acute changes in environmental temperature. Acute exposure to cold stress elicits cutaneous vasoconstriction, which is coupled with a marked increase in sympathetic nerve firing rate to the skin vascular

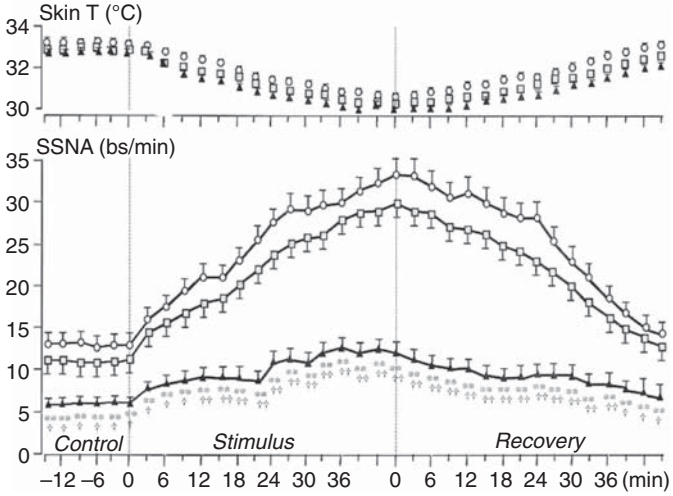

FIGURE 70.5. Effects of cold exposure on absolute skin temperature (skin T, upper panel) and on skin sympathetic nerve traffic (SSNA, lower panel) in young (open circles), middle-aged (closed squares), and elderly (triangles subjects). Data are shown as mean ± standard error of the mean (SEM) for the control normothermic period (control), during cold exposure (stimulus) and at the restoration of baseline temperature (recovery). Asterisks refer to the statistical significance between groups ($^{*\dagger}p < .05$; $^{**\dagger\dagger}p < .01$).

district (Fig. 70.5).[84] Conversely, heat stress triggers a pronounced cutaneous vasodilation, which in turn is linked to a significant decrease in skin sympathetic nerve traffic (Fig. 70.6).[84] Both the vascular and skin sympathetic neural responses appear to be altered in older individuals, the impairment representing one of the pathophysiologic hallmarks typical of the aging process (Figs. 70.5 and 70.6). [85]

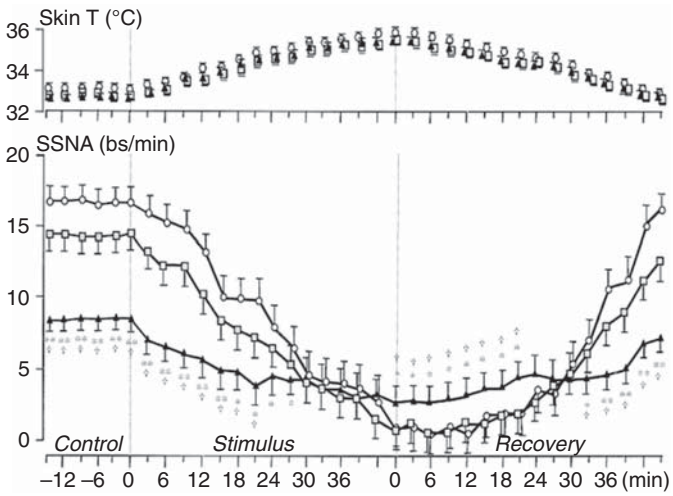

FIGURE 70.6. Effects of heat exposure on absolute skin temperature (skin T, upper panel) and on skin sympathetic nerve traffic (SSNA, lower panel) in young (open circles), middle-aged (closed squares), and elderly (triangles subjects). Data are shown as mean ± SEM for the control normothermic period (control), during cold exposure (stimulus) and at the restoration of baseline temperature (recovery). Asterisks and symbols refer to the statistical significance between groups ($^{*\dagger}p < .05$; $^{**\dagger\dagger}p < .01$).

Muscular Exercise

Muscular exercise constitutes the major recurrent normal stress on the cardiovascular system, and several reviews have emphasized the complexity of events that involve the regulation and integration of multiple systems, such as dilatation of the resistance vessels in the active muscle to provide the additional blood to meet their increased metabolic demand. The mechanisms are still unsettled after investigations that started in the 19th century. The muscle vasodilatation is accompanied by the appropriate increase in cardiac output. As exercise augments in severity, it is necessary to decrease the blood flow to other vascular beds to allow arterial blood pressure to increase. This is accomplished via the participation of the so-called central cardiovascular control mechanisms and local vascular control factors. The first ones refer to the central command, which integrates information stemming from arterial baroreceptors, cardiopulmonary receptors, as well as from ergoreceptors and metaboreceptors in the active muscle.[86] In addition, the so-called central command modulates autonomic outflow. The venous return, and hence the stroke volume, is sustained by the skeletal muscle pump and the reflex constriction of the splanchnic capacitance vessels. When static versus rhythmic exercise is performed, the so-called blood pressure-raising reflex is evoked from the active muscles.[86]

Although NO from the vascular endothelium may have a modest role in exercise hyperemia, in humans its presence is not essential for a nearly normal vasodilatation in skeletal muscles.[87] In the heart of the dog, it is estimated that NO is able to produce about one fourth of the coronary vasodilatation that occurred in response to exercise when all vasodilator systems were intact.[88] However, although NO production by the coronary circulation is increased with exercise, it does not affect levels of coronary blood flow, because it shifts the relationship between cardiac work and myocardial oxygen consumption, suggesting that endogenous NO modulates myocardial metabolism.[89] Chronic exercise in dogs increases eNOS gene expression, presumably by increasing endothelial shear stress, and this may contribute to the beneficial effects of sustained exercise on the cardiovascular system.[89]

Alterations in Cardiovascular Regulation in Pathologic States

Vasovagal Syncope

During vasovagal syncope, profound bradycardia and hypotension occur. Atropine administration can prevent the bradycardia but not the hypotension, suggesting that marked peripheral vasodilation is a major cause of the fall in arterial blood pressure. This concept has been confirmed, because vasovagal syncope can be seen in patients who have undergone heart transplantation and in patients subjected to cardiac pacing. In both cases, indeed, there is no bradycardia but there is hypotension during the syncopal attacks. The major site of the vasodilation is in the skeletal muscle district, and muscle sympathetic nerve activity is suppressed just before and during vasovagal attacks, indicating that sympathetic withdrawal contributes to the dilatation.

However, the skeletal muscle vasodilation seen during syncope is greater than that caused by sympathetic withdrawal alone, and it is absent in limbs that have undergone surgical sympathectomy or local anesthetic nerve block. These observations suggest a role for neurally mediated active vasodilation during syncope. The afferent neural pathways that evoke the profound vasodilation during vasovagal attacks remain to be identified. The neural pathways responsible for the active component of the dilation are also unknown. Recent evidence has demonstrated that cholinergic, β-adrenergic, and nitroxidergic (NO) vasodilator mechanisms are not essential for the dilation.[90]

Patients with orthostatic vasovagal reactions have impaired vagal baroreflex responses to arterial pressure changes below resting levels but normal initial responses to upright tilt. The final trigger of human orthostatic vasovagal reactions appears to be the abrupt disappearance of muscle sympathetic nerve activity.[91] Contrary to previous concepts, however, recent findings indicate that patients with a history of vasovagal syncope, compared to age-matched healthy controls, exhibit not only an increased resting peripheral sympathetic neural drive, but also impaired sympathoexcitatory responses to maneuvers capable of stimulating cardiopulmonary receptors.[92]

Smoking

Cigarette smoking has been historically known as a stimulus capable to markedly activate the sympathetic nervous function. This stimulation, however, has mainly a peripheral nature because (1) epinephrine and, to a lesser extent, norepinephrine release from the adrenal glands is markedly increased by the smoking act and (2) central sympathetic outflow is concomitantly inhibited during smoking, presumably because of a baroreflex activation brought about by the smoking-related pressor response.[93] Similar effects have been described during passive smoking.[94] Baroreflex sensitivity is impaired in habitual smokers, which may contribute to the smoking-related increase in arterial blood pressure and heart rate and to the decrease in heart rate variability during smoking.[95]

Long-term cigarette smoking is associated with an impaired endothelium-dependent coronary vasodilation regardless of the presence or absence of coronary atherosclerotic lesions,[96] the phenomenon probably being dependent on a nicotine-dependent deficiency in NO bioactivity.[97] The antioxidant vitamin C, as well as a potent reducing agent, tetrahydrobiopterin, improves endothelium-dependent responses in chronic smokers.[11,98] This observation supports the concept that endothelial dysfunction in chronic smokers is at least in part mediated by an enhanced production of oxygen-derived free radicals.[99] Coronary endothelial dysfunction also may occur in passive as well as in active smokers.[100]

Obesity and Obstructive Sleep Apnea

Human obesity is characterized by marked changes in the hemodynamic and metabolic states. In normotensive obese subjects, postganglionic sympathetic nerve firing rate to the leg muscles was twice that seen in lean control subjects.[101] This sympathetic activation is associated from a physiopathologic viewpoint with a number of abnormalities, which include (1) an arterial baroreflex dysfunction,[101] (2) a decrease in insulin sensitivity with a concomitant elevation of circulating insulin levels and consequent proexcitatory effects on central adrenergic drive,[102] and (3) an increase in plasma leptin levels that may also favor a hyperadrenergic state.[103] Other aspects of the sympathetic overactivity characterizing human obesity include the evidence that the neural abnormalities are more frequent in abdominal rather than in peripheral obesity[104] (Fig. 70.7) and appear to be markedly

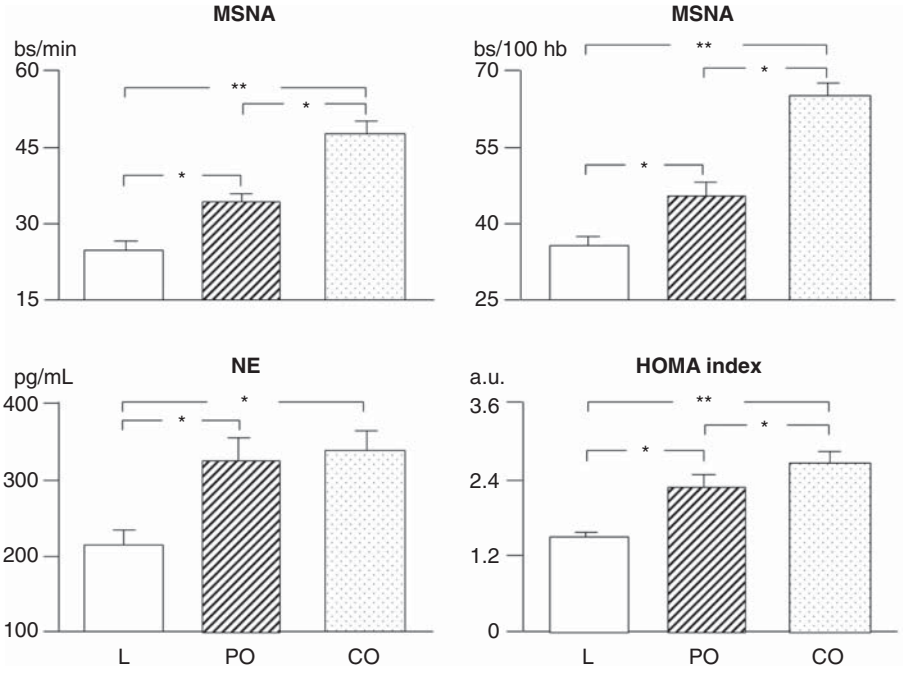

FIGURE 70.7. Muscle sympathetic nerve activity (MSNA) expressed as bursts incidence over time (bs/min) and corrected for heart rate (hb) (bs/100hb), venous plasma norepinephrine (NE) and homeostasis model assessment (HOMA) index in lean subjects (L) and in individuals with peripheral (PO) or central (CO) obesity. Data are expressed as mean ± SEM. Statistical significance between groups: $^{*}p < .05$; $^{**}p < .01$.

potentiated by the concomitance of hypertension and congestive heart failure,[105] that is, two cardiovascular diseases frequently complicating the obese state of marked clinical severity. Another frequent complication of obesity is the obstructive sleep apnea syndrome, which is capable of potentiating the sympathetic activation of the obese state,[106] thus exposing the patient to the adverse consequences (including the proarrhythmogenic ones) of a further hyperadrenergic activation.

In obese normotensive subjects, a reduction in body weight induced by a hypocaloric diet with normal sodium content exerts a marked reduction in sympathetic activity due to central sympathoinhibition.[107] This can be the consequence of an increased insulin sensitivity but also of a restoration of the baroreflex control of the cardiovascular system with weight loss. This has clinical implications because the removal of the sympathetic activation through weight loss has favorable effects on the high prevalence of hypertension, CHF, ischemic heart disease, and sudden death typical of obese people. The suppression of sympathetic activity associated with the correction of an overweight condition, however, may not have an entirely favorable significance because in obese subjects a sympathetic activation may favor energy consumption and thus oppose further body weight increase; its suppression by body weight loss may thus predispose to a weight regain ("weight cycling phenomenon").[107] The profound cardiovascular alterations characterizing the obese state are not confined to neural reflex abnormalities, however. Human obesity is also characterized by a well-documented endothelial dysfunction, which appears to be detectable in the early stages of the disease.[108] It is likely that the obesity-related metabolic abnormalities participate in the phenomenon.

Diabetes and Metabolic Syndrome

There appears to be diminished basal NO production in diabetes. Decreases in endothelium-dependent relaxation are common in both conduit and resistant arteries of chemically induced experimental diabetic animals. In humans, endothelial dysfunction was first reported in penile corpora cavernosa of patients with insulin-dependent and non–insulin-dependent diabetes mellitus, but it is still unclear whether the response of the vascular smooth muscle to NO is compromised.[109] The time of onset of these changes in the clinical course of the disease appears to have relevance. In addition, diabetes is associated with an enhanced production of endothelium-derived contracting factors derived from the cyclooxygenase pathway.

Experimental evidence supports the notion that hyperglycemia decreases endothelium-derived NO. Indeed when normal aortic rings are incubated in a hyperglycemic milieu, endothelium-dependent relaxation is impaired. Similarly, endothelium-dependent vasodilation is reduced in healthy subjects during a hyperglycemic clamp.[110] Hyperglycemia induces a series of cellular events that increase the production of reactive oxygen, thus inactivating NO to form peroxynitrite.[111] Hyperglycemia may initiate this process by increasing superoxidase anion production via the mitochondrial electron transport chain.[112] Another metabolic factor of key importance in diabetes mellitus is represented by insulin resistance. Insulin stimulates NO production from endothelial cells by increasing the activity of NOS via activation of specific kinases.[109] Thus while in healthy subjects insulin increases endothelium-dependent vasodilation, in insulin-resistant patients the insulin-dependent vasodilatory process is impaired.[109] Vitamin C selectively restores the impaired endothelium-dependent vasodilation in the forearm resistance vessels of patients with insulin-dependent diabetes mellitus.[113] Similarly, drug therapies that increase insulin sensitivity, such as metformin and thiazolidinediones, improve endothelium-dependent vasodilation.[109,114] These findings indicate that NO degradation by oxygen-derived free radicals contributes to abnormal vascular reactivity in humans with this disease.

The above-mentioned vascular and endothelial abnormalities of the diabetic state are associated with (and probably exacerbated by) alterations in sympathetic function. Evidence exists that these adrenergic abnormalities are of early occurrence in the disease development and potentiated by the concomitance of other cardiovascular diseases.[105,115] This is particularly the case for the so called metabolic syndrome, that is, a cluster of cardiovascular risk factors (such as hyperglycemia, insulin resistance, low high-density lipoprotein, blood pressure levels greater than 130/85 mm Hg, and visceral obesity) characterized by an endothelial dysfunction and by a marked sympathetic activation.[116]

Hypertension

Baroreceptor modulation of heart rate is impaired in patients with either essential or secondary hypertension, but there is controversy as to whether there are changes in the modulation of vasomotor tone due to the impairment of sympathetic control of systemic vascular resistance.[117] In moderate and more severe essential hypertensive patients, stimulation and deactivation of baroreceptors by alteration of arterial blood pressure through vasoactive drug infusions cause much less reflex bradycardia and tachycardia, respectively, than in age-matched normotensive subjects. However, the concomitant reflex inhibition and excitation of muscle sympathetic nerve traffic are superimposable in normotensive and hypertensive groups. Thus, in essential hypertension, the well-known impairment of the baroreflex ability to modulate the sinus node is not accompanied by any similar impairment of the baroreflex sympathetic modulation, which is of fundamental importance for the main baroreflex function (i.e., homeostatic blood pressure control).[118]

Concerning the role of NO, in studies with spontaneously hypertensive rats, it appears that the L-arginine–NO pathway in the rostral ventrolateral medulla is impaired and this may contribute to the increase in arterial pressure.[119] This translates in studies in the human forearm circulation into the evidence that release of NO is reduced in patients with uncomplicated essential hypertension.[120] In prehypertensive rats, dysfunctional constitutive nitric oxide synthase (cNOS) may contribute to the development of hypertension.[121] This also may explain why eNOS expression and function are increased rather than decreased despite normal endothelial function in spontaneously hypertensive rats.[122]

As mentioned previously, a cofactor, tetrahydrobiopterin, is required for activation of NOS and the release of NO.[11]

Tetrahydrobiopterin is also a potent reducing agent, and it is possible that prolonged oxidative stress may change the redox environment in endothelium and vascular smooth muscle cells, leading to a depletion of reduced tetrahydrobiopterin. As a consequence, there may be an impairment of NOS and hence of NO production.[123] Endothelial relaxation also is reduced because of endothelium-dependent production of vasoconstrictor prostanoids. Antihypertensive therapy improves endothelium-dependent hyperpolarization in spontaneously hypertensive rats.[124] In Sprague-Dawley rats, Ang II–mediated hypertension is associated with an enhanced production of ET-1 in vivo.[125] Endothelin-1 partially mediates Ang II–induced vascular changes in vivo. Studies with Wistar-Kyoto rats suggest that the angiotensin type I (AT1xxx) receptor antagonists, but not calcium antagonists, modulate tissue ET-1.[126] In the rat, chronic ET_A receptor blockade partially prevents Ang II–induced hypertension and the alterations in the endothelial function.[127]

In asymptomatic elderly hypertensive patients with the ACE DD genotype, it is speculated that the ACE D allele is a risk factor for the development of hypertension associated with endothelial cell damage.[128] Concerning the role of genetic versus environmental factors in essential hypertension, it has been shown that handgrip exercise resulted in a sustained ET-1 release into the bloodstream during recovery in the normotensive young male offspring of hypertensive parents compared with the offspring of normotensive parents.[129] The endothelial dysfunction observed in hypertensive blood vessels is likely to be a consequence rather than a cause of the disease process.[34]

In epidemiologic and clinical studies, essential hypertension has been correlated with insulin resistance and hyperinsulinemia in humans. However, the results of studies in obese hypertensive patients argue against the hypothesis of a casual pressor effect of insulin as the "missing link" between insulin resistance and essential hypertension.[130] In rats, AT1 receptors have a determinant role in the pathogenesis of insulin-induced hypertension.[131] It appears that endogenous dopamine and renal D_{1A} receptors have an important role in the regulation of sodium and body volume homeostasis. There is evidence that a defective renal dopaminergic receptor signaling system contributes to the development and maintenance of hypertension, but the nature of the defect in this system is unsolved.[132]

Heart Failure

In patients with CHF, baroreceptor regulation of the autonomic drive to the heart and systemic vessels is impaired. This dysfunction in part may account for the fact that sympathetic neural outflow is increased. Studies have shown that compared with age-matched control subjects, patients with mild symptoms of CHF and only a limited impairment of cardiac function display increased sympathetic nerve activity. This indicates that in this disease there is an early impairment of reflex sympathetic restraint, possibly as a consequence of a reduction in arterial compliance, with a resultant decreased responsiveness of the baroreceptors to pressure stimuli.[133] Evidence has been provided that the sympathetic activation (1) has prognostic relevance, (2) is linked to the insulin resistance state typical of the disease itself, and (3) is similar regardless of the ischemic or idiopathic nature of the disease (Fig. 70.8).[134] Chronic ACE inhibitor treatment is accompanied by a marked reduction in central sympathetic outflow. This reduction may depend on a persistent restoration of baroreflex restraint on the sympathetic neural drive.[135] Similar sympathoinhibitory effects have been shown during long-term transdermal clonidine administration.[136]

In the peripheral resistance arteries in patients with CHF, an endothelial dysfunction has been documented. There is an impaired flow-dependent, endothelium-mediated dilation

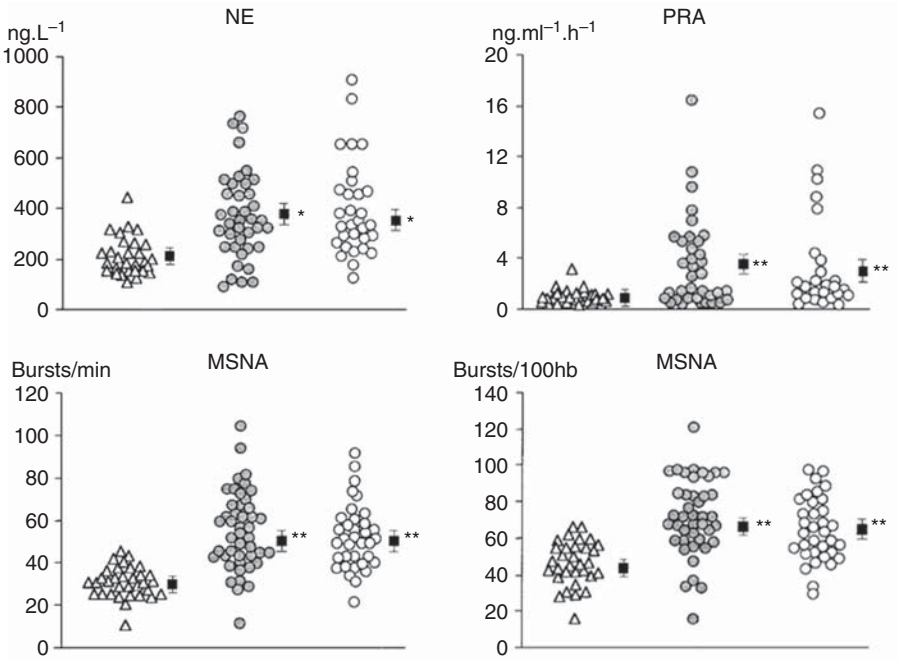

FIGURE 70.8. Individual and mean values of plasma norepinephrine (NE), plasma renin activity (PRA), and muscle sympathetic nerve traffic (MSNA) expressed as bursts incidence over time (bursts/min) and corrected for heart rate values (bursts/100 heart beats) in control subjects (triangles) and in patients with congestive heart failure due to ischemic heart disease (IHD, black circles) or idiopathic dilated cardiomyopathy (IDC, white circles). NE and MSNA values were always significantly different (*$p <$ .05; **$p <$ .01) between patients with CHF and control subjects, but not between patients with IHD and IDC.

of conduit arteries, and the formation and basal release of NO are decreased in the coronary circulation in the absence of coronary artery disease.[137] Physical training restores endothelium-mediated flow-dependent dilatation in these patients, possibly via enhanced endothelial release of NO, while the improvement associated with vitamin C administration results from an increased availability of NO.[138,139] Endothelin may participate in the adaptations to acute reductions in perfusion pressure in CHF. The kidney, lung, heart, and peripheral vasculature are sites of ET mRNA expression that may contribute to its elevation. The mechanisms contributing to its increase in CHF probably include increased atrial and venous pressures and reduced perfusion pressure and shear stress. The vasoconstrictor action of ET may be beneficial in the early phases of CHF, augmenting cardiac preload via venoconstriction and increasing systemic vascular resistance to guarantee an adequate perfusion pressure despite the counteraction of NO and atrial natriuretic peptides. However, as the heart failure progresses, the increasing action of ET contributes to the deterioration of cardiac function.[138,139] Concerning the role of ET receptors in CHF, ET_A receptors exert a pathophysiologic vasoconstrictive effect, whereas ET_B receptors generally have a vasodilative action. Hence, for the treatment of CHF, it seems that a selective ET_A receptor antagonist may offer therapeutic benefits.[138,139]

Hypercholesterolemia

The induction of hypercholesterolemia in animals with high-fat or high-cholesterol diets impairs endothelium-dependent relaxations. This impairment is also seen in genetically hyperlipidemic rabbits and in humans with atherosclerosis, hypercholesterolemia or both.[34] Interestingly, the endothelial dysfunction associated with hypercholesterolemia appears to be manifest even after an acute high-fat meal, a finding suggesting the fast development of the adverse effects of this metabolic condition on the vascular function.[140] In the pig, experimental hypercholesterolemia is characterized by an enhanced coronary vasoconstriction and an attenuated NO activity. This is associated with a decrease in eNOS immunoreactivity without a change in ET receptor density or binding affinity.[141]

Atherosclerosis

Oxidized low-density lipoproteins (LDLs) at high plasma concentrations are a major risk factor for the development of atherosclerosis. They may contribute to this via various mechanisms, including being a chemoattractive substance for monocytes, enhancing lipid accumulation by monocytes, impairing metabolic activity of vascular cells, and altering endothelial function. Concerning the latter, in addition to causing vasodilatation, NO inhibits platelet adherence and aggregation, vascular smooth muscle proliferation, and endothelial cell-leukocyte interactions. Thus, a reduction in NO synthesis in endothelial cells or in its release may be involved in the development of atherosclerosis. It seems that an oxidized form of LDL specifically impairs endothelium-dependent vasodilatation by reducing NO synthesis through enhanced production of superoxide anion and a consequent reduction in the cellular level of L-arginine.[142] This may explain why eNOS expression, as well as NO production, is reduced in atherosclerotic arteries.[143]

Gene Transfer

Gene therapy involves the transfer of a functional gene into host cells to correct the malfunction of a specific gene or to alleviate the symptoms of a disease. Vascular gene transfer refers to the introduction of genes into relevant cells of the blood vessels wall. For gene transfer to the cardiovascular system, adenoviral vectors are the most efficient means of transfer.[144]

The eNOS genes have been expressed in dog basilar arteries by a replication-incompetent adenovirus. The expression of recombinant eNOS in endothelial cells may prove useful in the site-specific therapy of vascular diseases characterized by endothelial dysfunction, such as atherosclerosis and hypertension. In addition, adventitial fibroblasts differentiate into myofibroblasts and migrate into the intima. Because recombinant eNOS can be targeted to these fibroblasts, this might inhibit cellular proliferation.[144]

In pigs, percutaneous adenovirus-mediated NOS gene transfer after angioplasty in coronary arteries restored NO production. In canine basilar arteries affected by subarachnoid hemorrhage, after successful eNOS gene transfer to the spastic vessel, the impaired NO-mediated relaxation was partially restored through the local (adventitial) production of NO.[145] In humans, intimal proliferation in the veins is due to smooth muscle cells from these vessels, in which platelet-derived growth factor increases mitogen-activated protein kinase in vein muscle and downregulates cell cycle inhibitor in the vein muscle, not in the artery muscle. These findings may contribute to the longer patency of arterial versus venous grafts.[146] The question may be asked as to whether eNOS gene transfer in the latter would improve their patency. To add to the complexity, functional thrombin receptors are present on the endothelium and smooth muscle cells of human coronary bypass vessels, both internal mammary artery and saphenous vein. These receptors on the endothelium mediate relaxation in the artery but not in the vein. In addition, thrombin causes greater contraction and proliferation in the smooth muscle cells of the saphenous vein.[147] A deletion polymorphism in the ACE gene is associated with a high serum level of ACE, with the resultant risk of myocardial infarction. Angiotensin-like receptors include at least two different subtypes: AT1 and AT2. In Sprague-Dawley rats, antisense oligodeoxynucleotides directed at AT1 receptors mRNA prevented an increase in plasma Ang II immediately after ischemia-reperfusion.[148,149]

Summary

Basic and clinical studies performed in the past decade have facilitated remarkable expansion in our knowledge of the complex pathophysiologic background characterizing cardiovascular diseases in human beings. Evidence has also been provided that complex but crucial relationships among metabolic, humoral, neural, and hemodynamic factors take place in different pathologic states, affecting both blood vessels and cardiac muscle performance and structure. These

relationships represent the target for specific pharmacologic interventions aimed at reducing cardiovascular morbidity and improving cardiovascular health.

References

1. Brunner H, Cockcroft JR, Deanfield J, for the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. J Hypertens 2005;23:233–246.
2. Spieker LE, Luscher TF. Endothelium in hypertension: nitric oxide. In: Oparil S, Weber MA, eds. Hypertension. New York: Elsevier Saunders, 2005:146–154.
3. Cosentino F, Katusic ZS. Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. Circulation 1995;91:139–144.
4. Davies PF. Flow-mediated endothelial mechanotransduction. Physiol Rev 1995;75:519–560.
5. Ziegler T, Silacci P, Harrison VJ, Hayoz D. Nitric oxide synthase expression in endothelial cells exposed to mechanical forces. Hypertension 1998;32:351–355.
6. Gudi SR, Clark CB, Frangos JA. Fluid flow rapidly activates G proteins in human endothelial cells. Involvement of G proteins in mechanochemical signal transduction. Circ Res 1996;79: 834–839.
7. Pou S, Pou WS, Bredt DS, et al. Generation of superoxide by purified brain nitric oxide synthase. J Biol Chem 1992;267: 24173–24176.
8. Hishikawa K, Luscher TF. Pulsatile stretch stimulates superoxide production in human aortic endothelial cells. Circulation 1997;96:3610–3616.
9. Wyss CA, Koepfli P, Namdar M, et al. Tetrahydrobiopterin restores impaired coronary microvascular dysfunction in hypercholesterolaemia. Eur J Nucl Med Mol Imaging 2005;32: 84–91.
10. Katusic ZS, Cosentino F. Nitric oxide synthase: from molecular biology to cerebrovascular physiology. News Physiol Sci 1994;9: 64–67.
11. Cosentino F, Luscher TF. Tetrahydrobiopterin and endothelial function. Eur Heart J 1998;19(suppl G):G3–8.
12. Wang HD, Pagano PJ, Du Y, Cayatte AJ, Quinn MT, Brecher P, Cohen RA. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. Circ Res 1998;82: 810–818.
13. Spieker LE, Ruschitzka F, Luscher TF, et al. HDL and inflammation in atherosclerosis. Curr Drug Targets Immune Endocr Metab Disord 2004;4:51–57.
14. Hishikawa K, Oemar BS, Yang Z, et al. Pulsatile stretch stimulates superoxide production and activates nuclear factor-kB in human coronary smooth muscle. Circ Res 1997;81:797–803.
15. Creager MA, Roddy MA, Boles K, et al. N-Acetylcysteine does not influence the activity of endothelium-derived relaxing factor in vivo. Hypertension 1997;29:668–672.
16. Martin CM, Beltran-Del Rio A, Albrecht A, et al. Local cholinergic mechanisms mediate nitric oxide-dependent flow-induced vasorelaxation in vitro. Am J Physiol 1996;270:H442–H446.
17. Miller VM, Vanhoutte PM. Endothelial α_2-adrenoreceptors in canine pulmonary and systemic blood vessels. Eur J Pharmacol 1985;118:123–129.
18. Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314–1319.
19. Laycock SK, Vogel T, Forfia PR, et al. Role of nitric oxide in the control of renal oxygen consumption and the regulation of chemical work in the kidney. Circ Res 1998;82:1263–1271.
20. Shen W, Hintze TH, Wolin MS. Nitric oxide. An important signaling mechanism between vascular endothelium and parenchymal cells in the regulation of oxygen consumption. Circulation 1995;92:3505–3512.
21. Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. Circ Res 1996;79:363–380.
22. Cooper CJ, Landzberg MJ, Anderson TJ, et al. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. Circulation 1996;93:266–271.
23. Popp R, Fleming I, Busse R. Pulsatile stretch in coronary arteries elicits release of endothelium-derived hyperpolarizing factor: a modulator of arterial compliance. Circ Res 1998;82: 696–703.
24. Barton M, Beny JL, d'Uscio LV, et al. Endothelium-independent relaxation and hyperpolarization to C-type natriuretic peptide in porcine coronary arteries. J Cardiovasc Pharmacol 1998;31: 377–383.
25. Luscher TF. Endothelial dysfunction: the role and impact of the renin-angiotensin system. Heart 2000;84:20–22.
26. Taddei S, Grassi G. Angiotensin II as the link between nitric oxide and neuroadrenergic function. J Hypertens 2005;23: 935–937.
27. Parris RJ, Webb DJ. The endothelin system in cardiovascular physiology and pathophysiology. Vasc Med 1997;2:31–43.
28. Cannan CR, Burnett JC Jr, Brandt RR, et al. Endothelin at pathophysiological concentrations mediates coronary vasoconstriction via the endothelin-A receptor. Circulation 1995;92: 3312–3317.
29. Haynes WG, Ferro CJ, O'Kane KPJ, et al. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation 1996;93:1860–1870.
30. Spieker LE, Luscher TF, Noll G. ETA receptors mediate vasoconstriction of large conduit arteries during reduced flow in humans. J Cardiovasc Pharmacol 2003;42:315–318.
31. Verhaar MC, Strachan FE, Newby DE, et al. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation 1998;97:752–756.
32. Traverse JH, Judd D, Bache RJ. Dose-dependent effect of endothelin-1 on blood flow to normal and collateral-dependent myocardium. Circulation 1996;93:558–566.
33. Filep JG. Endogenous endothelium modulates blood pressure, plasma volume and albumin escape after systemic nitric oxide blockade. Hypertension 1997;30:22–28.
34. Vanhoutte PM, Feletou M, Taddei S. Endothelium-dependent contraction in hypertension. Br J Pharmacol 2005;144:449–458.
35. Burnstock G, Ralevic V. Cotransmission. In: Garlad CJ, Angus J, eds. The Pharmacology of Smooth Muscle. Oxford: Oxford University Press, 1996.
36. Westfall TC, Yang CL, Curfam-Falvey M. Neuropeptide-Y-ATP interactions at the vascular sympathetic neuroeffector junction. J Cardiovasc Pharmacol 1995;26:682–687.
37. Potter EK, Ulman LG. Neuropeptides in sympathetic nerves affect vagal regulation of the heart. New Physiol Sci 1994;9: 174.
38. Aliev G, Ralevic V, Burnstock G. Depression of endothelial nitric oxide synthase but increased expression of endothelin-1 immunoreactivity in rat thoracic aortic endothelium associated with long-term, but not short-term, sympathectomy. Circ Res 1996;79:317–323.
39. Okamura T, Yoshida K, Toda N. Nitroxidergic innervation in dog and monkey renal arteries. Hypertension 1995;25:1090–1095.
40. Toda N, Okamura T. Nitroxidergic nerve: regulation of vascular tone and blood flow in the brain. J Hypertens 1996;14: 423–434.

41. Henriksen O. Circulatory studies: local sympathetic venoarteriolar axon "reflex" in the sympathoadrenal system. In: Christensen NJ, Henriksen O, Lassen LA, eds. The Symapthoadrenal System: Physiology and Pathophysiology. Copenhagen: Munksgaard, 1986:67–80.
42. Stoyneva Z. Laser doppler-recorded venoarterial reflex in Raynaud's phenomenon. Auton Neurosci 2004;116:62–68.
43. Grassi G, Colombo M, Seravalle G, et al. Dissociation between muscle and skin sympathetic nerve activity in essential hypertension, obesity and congestive heart failure. Hypertension 1998;31:64–67.
44. Mancia G, Grassi G. Mechanisms and clinical implications of blood pressure variability. J Cardiovasc Pharmacol 2000;35(suppl 4):S15–S19.
45. Grassi G, Seravalle G, Calhoun DA, Mancia G. Physical training and baroreceptor control of sympathetic nerve activity in humans. Hypertension 1994;23:294–301.
46. Hironaga K, Hirooka Y, Matsuo I, et al. Role of endogenous nitric oxide in the brain stem on the rapid adaptation of baroreflex. Hypertension 1998;31:27–31.
47. Liu JL, Murakami H, Zucker IH. Angiotensin II-nitric oxide interaction on sympathetic outflow in conscious rabbits. Circ Res 1998;82:496–502.
48. van der Linde NAJ, Boomsma F, van den Meiracker AH. Role of nitric oxide in modulating systemic pressor responses to different vasoconstrictors in man. J Hypertens 2005;23:1009–1015.
49. Mancia G, Grassi G, Ferrari A. Reflex control of the circulation in experimental and human hypertension. In: Zanchetti A, Mancia G, eds. Handbook of Hypertension: Pathophysiology of Hypertension. Amsterdam: Elsevier, 1997:568–613.
50. Rand MJ, Li CG. Nitric oxide as a neurotransmitter in peripheral nerves: nature of transmitter and mechanism of transmission. Annu Rev Physiol 1995;57:659–682.
51. Shepherd JT. Perivascular nerves and endothelial cells: normal actions and interactions and changes in hypertension. High Blood Press 1996;5:124–138.
52. Feigl EO. Neural control of coronary blood flow. J Vasc Res 1998;35:85–92.
53. Radelli A, Mircoli L, Perini S, et al. Lack of autonomic contributions to tonic nitric oxide-mediated vasodilatation in unanesthetized free-moving rats. J Hypertens 1998;16:55–61.
54. Grassi G. Renin-angiotensin-sympathetic crosstalks in hypertension: reappraising the relevance of peripheral interactions. J Hypertens 2001;19:1713–1716.
55. Hennington BS, Zhang H, Miller MT, et al. Angiotensin II stimulates synthesis of endothelial nitric oxide synthase. Hypertension 1998;31:283–288.
56. Perondi R, Saino A, Tio RA, et al. ACE inhibition attenuates sympathetic coronary vasoconstriction in patients with coronary artery disease. Circulation 1992;85:2004–2013.
57. Saino A, Pomidossi G, Perondi R, et al. Modulation of sympathetic coronary vasoconstriction by cardiac renin-angiotensin system in human coronary artery disease. Circulation 2000; 101:2277–2283.
58. Rubanyi GM, Johns A, Kauser K. Effect of estrogen on endothelial function and angiogenesis. Vasc Pharmacol 2002;38:89–98.
59. Chambliss KL, Shaul PW. Estrogen modulation of endothelial nitric oxide synthase. Endocr Rev 2002;23:665–686.
60. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. Am J Physiol 2004;286:R233–R249.
61. Kleinert H, Wallerath T, Euchenhofer C, et al. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. Hypertension 1998;31:582–588.
62. Davidge ST, Zhang Y. Estrogen replacement suppresses a prostaglandin H synthase-dependent vasoconstrictor in rat mesenteric arteries. Circ Res 1998;83:388–395.
63. Virdis A, Ghiadoni L, Pinto S, et al. Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. Circulation 2000;101:2258–2263.
64. Tolbert T, Oparil S. Cardiovascular effects of estrogen. Am J Hypertens 2001;14:186S–193S.
65. Szokodi I, Kinnunen P, Tavi P, et al. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 1998;97:1062–1070.
66. Creager MA, Liang CS, Coffman JD. Beta adrenergic-mediated vasodilator response to insulin in the human forearm. J Pharmacol Exp Ther 1985;235:709–714.
67. Wu HY, Jeng YY, Yue CJ, et al. Endothelial-dependent vascular effects of insulin and insulin-like growth factor I in the perfused rat mesenteric artery and aortic ring. Diabetes 1994;43: 1027–1032.
68. Scherrer U, Randin D, Vollenweider P, et al. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 1994;94:2511–2515.
69. Hasdai D, Holmes DR Jr, Richardson DM, et al. Insulin and IGF-I attenuate the coronary vasoconstrictor effects of endothelin-1 but not of sarafotoxin 6c. Cardiovasc Res 1998;39:644–650.
70. Folkow B, Svanborg A. Physiology of cardiovascular aging. Physiol Rev 1993;73:725–764.
71. Ferrari AU, Radaelli A, Centola M. Aging and the cardiovascular system. J Appl Physiol 2003;95:2591–2597.
72. Davy KP, Seals DR, Tanaka H. Augmented cardiopulmonary and integrative sympathetic baroreflexes but attenuated peripheral vasoconstriction with age. Hypertension 1998;32:298–304.
73. Jones PP, Christon DD, Jordan J, et al. Baroreflex buffering is reduced with age in healthy men. Circulation 2003;107:1770–1774.
74. Davy KP, Tanaka H, Andros EA, et al. Influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult humans. Am J Physiol 1998;275:H1768–1772.
75. Gerhard M, Roddy MA, Creager SJ, et al. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension 1996;27:849–853.
76. Seals DR, Bell C. Chronic sympathetic activation: consequence and cause of age-associated obesity. Diabetes 2004;53:276–284.
77. Esler M, Hasting J, Lambert G, et al. The influence of aging of the human sympathetic nervous system and brain norepinephrine turnover. Am J Physiol 2002;282:R909–R916.
78. Tschudi MR, Barton M, Bersinger NA, et al. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. J Clin Invest 1996;98:899–905.
79. Cernadas MR, Sanchez de Miguel L, Garcia-Duran M, et al. Expression of constitutive and inducible nitric oxide synthases in the vascular wall of young and aging rats. Circ Res 1998;83: 279–286.
80. Wieling W, Shepherd JT. Initial and delayed circulatory responses to orthostatic stress in normal humans and in subjects with orthostatic intolerance. Int Angiol 1992;11:69–82.
81. Noll G, Wenzel RR, Schneider M, et al. Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. Circulation 1996;93:866–869.
82. Dietz NM, Rivera JM, Eggener SE, et al. Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. J Physiol 1994;480:361–368.
83. Spieker LE, Hurlimann D, Ruschitzka F, et al. Mental stress induces prolonged endothelial dysfunction via endothelin-A receptors. Circulation 2002;105:2817–2820.
84. Grassi G, Seravalle G, Turri C, et al. Impairment of thermoregulatory control of skin sympathetic nerve traffic in the elderly. Circulation 2003;108:729–735.

85. Kenney WL, Munce TA. Aging and human temperature regulation. J Appl Physiol 95:2598–2603.
86. Delp MD, O'Leary DS. Integrative control of the skeletal muscle circulation in the maintenance of arterial pressure during exercise. J Appl Physiol 2004;97:1112–1118.
87. Rowell LB. Ideas about control of skeletal and cardiac muscle blood flow (1876–2003): cycles of revision and new vision. J Appl Physiol 2004;97:384–392.
88. Bernstein RD, Ochoa FY, Xu X, et al. Function and production of nitric oxide in the coronary circulation of the conscious dog during exercise. Circ Res 1996;79:840–848.
89. Sessa WC, Pritchard K, Seyedi N, et al. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circ Res 1997;74:349–353.
90. Dietz NM, Joyner MJ, Shepherd JT. Vasovagal syncope and skeletal muscle vasodilatation: the continuing conundrum. Pacing Clin Electrophysiol 1997;20:775–780.
91. Morillo CA, Eckberg DL, Ellenbogen KA, et al. Vagal and sympathetic mechanisms in patients with orthostatic vasovagal syncope. Circulation 1997;96:2509–2513.
92. Bechir M, Binggeli C, Corti R, et al. Dysfunctional baroreflex regulation of sympathetic nerve activity in patients with vasovagal sincope. Circulation 2003;107;1620–1625.
93. Grassi G, Seravalle G, Calhoun DA, et al. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. Circulation 1994;90:248–253.
94. Narkiewicz K, van de Borne PJ, Hausberg M, et al. Cigarette smoking increases sympathetic outflow in humans. Circulation 1998;98:528–534.
95. Mancia G, Groppelli A, Di Rienzo M, et al. Smoking impairs baroreflex sensitivity in humans. Am J Physiol 1997;42:H1555–H1560.
96. Zeiher AM, Schachinger V, Minner J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilatorfunction. Circulation 1995;92:1094–1100.
97. Puranik R, Celermajer DS. Smoking and endothelial function. Prog Cardiovasc Dis 2003;45:443–458.
98. Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. Prog Cardiovasc Dis 2003;46:91–111.
99. Burke A, Fitzgerald GA. Oxidative stress and smoking-induced vascular injury. Prog Cardiovasc Dis 2003;46:79–90.
100. Anazawa T, Dimayuga PC, Li H, et al. Effect of exposure to cigarette smoke on carotid artery intimal thickening: the role of inducible NO synthase. Arterioscler Thromb Vasc Biol 2004; 24:1652–1658.
101. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation in obese normotensive subjects. Hypertension 1995;25: 560–563.
102. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension. J Hypertens 2001;19:523–528.
103. Grassi G. Leptin, sympathetic nervous system, and baroreflex function. Curr Hypertens Rep 2004;6:236–240.
104. Grassi G, Dell'Oro R, Facchini A, et al. Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives. J Hypertens 2004;22:2363–2369.
105. Grassi G, Seravalle G, Quarti-Trevano F, et al. Effects of hypertension and obesity on the sympathetic activation of heart failure patients. Hypertension 2003;42:873–877.
106. Narkiewicz K, van de Borne PJH, Cooley RL, et al. Sympathetic activity in obese subjects with and without obstructive sleep apnea. Circulation 1998;98:772–776.
107. Grassi G, Seravalle G, Colombo M, et al. Body weight reduction, sympathetic nerve traffic, and arterial baroreflex in obese normotensive humans. Circulation 1998;97:2037–2042.
108. Rahmouni K, Correia ML, Haynes WG, et al. Obesity-associated hypertension: new insights into mechanisms. Hypertension 2005;45:9–14.
109. Creager MA, Luscher TF, Cosentino F, et al. Diabetes and vascular disease. Circulation 2003;108:1527–1532.
110. Williams SB, Goldfine AB, Timimi FK, et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation 1998;97:1695–1701.
111. Beckman JA, Goldfine AB, Gordon MB, et al. Ascorbate restores endothelium dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 2001;103:1618–1623.
112. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks free pathways of hyperglycaemic damage. Nature 2000;404:787–790.
113. Timimi FK, Ting HH, Haley EA, et al. Vitamin C improves endothelial-dependent vasodilation in patients with insulin-dependent diabetes mellitus. J Am Coll Cardiol 1998;31:552–571.
114. Watanabe Y, Sunayama S, Shimada K, et al. Troglitazone improves endothelial dysfunction in patients with insulin resistance. J Atheroscler Thromb 2000;7:159–163.
115. Huggett RJ, Scott EM, Gilbey SG, et al. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. Circulation 2003;108:3097–3101.
116. Grassi G, Dell'Oro R, Quarti-Trevano F, et al. Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. Diabetologia 2005;48:1359–1365.
117. Grassi G, Cattaneo BM, Seravalle G, et al. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 1998;31:68–72.
118. Grassi G. Sympathetic and baroreflex function in hypertension: implications for current and new drugs. Curr Pharm Des 2004;10:3579–3589.
119. Kagiyama S, Tsuchihashi T, Abe I, et al. Enhanced depressor response to nitric oxide in the rostral ventrolateral medulla of spontaneously hypertensive rats. Hypertension 1998;31:1030–1034.
120. Linder L, Kiowski W, Buhler FR, et al. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in essential hypertension. Circulation 1990;81:1762–1767.
121. Cosentino F, Patton S, d'Uscio LV, et al. Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest 1998;101:1530–1537.
122. Nava E, Farré AL, Moreno C, et al. Alterations to the nitric oxide pathway in the spontaneously hypertensive rat. J Hypertens 1998;16:609–615.
123. Kinoshita H, Tsutsui M, Milstien S, et al. Tetrahydrobiopterin, nitric oxide and regulation of cerebral arterial tone. Prog Neurobiol 1997;52:295–302.
124. Onaka U, Fujii K, Abe I, et al. Antihypertensive treatment improves endothelium-dependent hyperpolarization in the mesenteric artery of spontaneously hypertensive rats. Circulation 1998;98:175–182.
125. Rajagopalan S, Laursen JB, Borthayre A, et al. Role for endothelin-1 in angiotensin II-mediated hypertension. Hypertension 1997;30:29–34.
126. d'Uscio LV, Shaw S, Barton M, et al. Losartan but not verapamil inhibits angiotensin II induced tissue endothelin 1 increase: role of blood pressure and endothelial function. Hypertension 1998;31:1305–1310.
127. d'Uscio LV, Moreau P, Shaw S, et al. Effects of chronic ETA-receptor blockade in angiotensin II-induced hypertension. Hypertension 1997;29:435–441.
128. Kario K, Matsuo T, Kobayashi H, et al. Endothelial cell damage and angiotensin-converting enzyme insertion/deletion genotype in elderly hypertensive patients. J Am Coll Cardiol 1998; 32:444–450.

129. Mangieri E, Tanzilli G, Barilla F, et al. Handgrip increases endothelin-1 secretion in normotensive young male offspring of hypertensive parents. J Am Coll Cardiol 1998;31:1362–1366.
130. El-Atat FA, Stas SN, McFarlane SI, et al. The relationship between hyperinsulinemia, hypertension and progressive renal disease. J Am Soc Nephrol 2004;15:2816–2827.
131. Fang TC, Huang WC. Angiotensin receptor blockade blunts hyperinsulinemia-induced hypertension in rats. Hypertension 1998;32:235–242.
132. Ferro A. Renal dopamine receptors and hypertension. J Hypertens 2003;21:37–38.
133. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. Circulation 1995;92:3206–3211.
134. Grassi G, Seravalle G, Bertinieri G, et al. Sympathetic and reflex abnormalities in heart failure secondary to ischaemic or idiopathic dilated cardiomyopathy. Clin Sci 2001;101:141–146.
135. Grassi G, Cattaneo BM, Seravalle G, et al. Effects of chronic ACE inhibition on sympathetic nerve traffic and baroreflex control of circulation in heart failure. Circulation 1997;96:1173–1179.
136. Grassi G, Turri C, Seravalle G, et al. Effects of chronic clonidine administration on sympathetic nerve traffic and baroreflex function in heart failure. Hypertension 2001;38:286–291.
137. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol 2004;15:1983–1992.
138. Drexler H, Hornig B. Endothelial dysfunction in human disease. J Mol Cell Cardiol 1999;31:51–60.
139. Bauersachs J, Schafer A. Endothelial dysfunction in heart failure: mechanisms and therapeutic approaches. Curr Vasc Pharmacol 2004;2:115–124.
140. Giannattasio C, Zoppo A, Gentile G, et al. Acute effect of high-fat meal on endothelial function in moderately dyslipidemic subjects. Arterioscler Thromb Vasc Biol 2005;25:406–410.
141. Verghese M, Cannan CR, Miller VM, et al. Enhanced endothelin-mediated coronary vasoconstriction and attenuated basal nitric oxide activity in experimental hypercholesterolemia. Circulation 1997;96:1930–1936.
142. Hein TW, Kuo L. LDLs impair vasomotor function of the coronary microcirculation: role of superoxide anions. Circ Res 1998;83:404–414.
143. Ozaki M, Kawashima S, Yamashita T, et al. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. J Clin Invest 2002;110:331–340.
144. Rissanen TT, Rutanen J, Yla-Herttuala S. Gene transfer for therapeutic vascular growth in myocardial and peripheral ischemia. Adv Genet 2004;52:117–164.
145. Onoue H, Tsutsui M, Smith L, et al. Expression and function of recombinant endothelial nitric oxide synthase gene in canine basilar artery after experimental subarachnoid hemorrhage. Stroke 1998;29:1959–1965.
146. Yang Z, Oemar BS, Carrel T, et al. Different proliferative properties of smooth muscle cells of human arterial and venous bypass vessels: role of PDGF receptors, mitogen-activated protein kinase, and cyclin-dependent kinase inhibitors. Circulation 1998;97:181–187.
147. Yang Z, Ruschitzka F, Rabelink TJ, et al. Different effects of thrombin receptor activation on endothelium and smooth muscle cells of human coronary bypass vessels. Implications for venous bypass graft failure. Circulation 1997;95:1870–1876.
148. Oudot A, Vergely C, Ecarnot-Laubriet A, et al. Pharmacological concentration of angiotensin-(1–7) activates NADPH oxidase after ischemia-reperfusion in rat heart through AT1 receptor stimulation. Regul Pept 2005;127:101–110.
149. Akao M, Sakurai T, Horie M, et al. Angiotensin II type 1 receptor blockade abolishes specific K(ATP) channel gene expression in rats with myocardial ischemia. J Mol Cell Cardiol 2000;32:2239–2247.