Cardiovascular Autonomic Imbalance and Baroreflex Dysfunction in the Apolipoprotein E-deficient Mouse

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Key Words

Hypercholesterolemia • Atherosclerosis • Mouse • Baroreflex function • Autonomic nervous system

Abstract

Genetically engineered mouse models and advances in molecular biotechnology have given extensive aid to experimental studies of cardiovascular mechanisms and dysfunction in pathological states such as atherosclerosis. Among the available animal models that have been developed to study atherosclerosis, the apolipoprotein E-deficient (apoE^{-/-}) mouse is the most ideal genetically modified animal presently available. The apoE^{-/-} mouse develops spontaneous severe hypercholesterolemia in a short-time and subsequently develops atherosclerotic lesions similar to those found in humans. Since its creation two decades ago, the apoE^{-/-} mouse has greatly contributed to the understanding of atherosclerosis, but the consequences of hypercholesterolemia and atherosclerosis for the autonomic control of cardiovascular function in this mouse model have not been reviewed. In this article, we provide an overview of abnormalities of the parasympathetic and sympathetic nervous systems controlling heart rate

and blood pressure and emphasize the dysfunction of the baroreflex control of cardiovascular function and how this dysfunction is influenced by nitric oxide, reactive oxygen species, aging and an atherogenic diet in the apoE^{-/-} mouse.

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Introduction

The mouse is an important experimental model for the study of genetic contributions to diseases due to its low cost of maintenance, easy breeding, short reproduction cycle, and the availability of inbred strains. A major disadvantage of the mouse model is its small size, which makes it relatively difficult to perform neural and hemodynamic measurements. However, advances in surgical techniques have overcome the size limitations of the mouse, making it possible to study cardiac [1, 2], vascular [3, 4] and renal [5] function in this animal.

Genetic discoveries and recent advances in genetic and molecular biotechnologies have provided the opportunity to develop many mouse models for human diseases including atherosclerosis, which is a multifactorial,

long-lasting process that affects critical organs in humans. Two decades ago, the first line of gene targeted murine models of atherosclerosis was created by inactivation of the apolipoprotein E (apoE) gene by homologous recombination [6-8]. Among the available genetically engineered models, the apoE-deficient (apoE^{-/-}) mouse is considered to be one of the most relevant models because it develops spontaneous hypercholesterolemia and arterial lesions similar to those observed in humans.

The apoE^{-/-} mouse model has provided many opportunities to study the fundamental molecular processes that underlie the physiological mechanisms and their dysregulation in the atherosclerotic disease. However, the effects of hypercholesterolemia and atherosclerosis on the autonomic nervous control of cardiac and vascular function in the apoE^{-/-} mouse have not yet been reviewed. Here, we first present an overview of plasma lipid profiles and the progression of atherosclerosis in the apoE^{-/-} mouse. We then focus on new findings obtained during the last several years, including findings obtained in our own laboratory and in collaboration with Mark W. Chapleau's laboratory (The University of Iowa) regarding the neurohumoral and autonomic nervous control of heart rate (HR) and blood pressure (BP), which serve to aid in the discussion of the underlying mechanisms involved in the neural baroreflex control of cardiovascular function in the apoE^{-/-} mouse model.

Lipoprotein and atherosclerosis phenotypes

As summarized in Figure 1, in the apoE^{-/-} mouse, most of the plasma cholesterol (PC) is in atherogenic lipoprotein fractions, including very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL), and this profile is aggravated by a Western-type diet. ApoE^{-/-} mice in comparison with C57BL/6J (C57) genetic background mice have increased total PC, triglycerides (TG), and VLDL+IDL. When fed a Western-type diet, the proportions of these lipids dramatically increase in total PC and particularly in the VLDL+IDL lipoprotein fraction [7, 9-15]. Figure 1 summarizes the progression of atherosclerotic lesions throughout the conducting macrovasculature, including sites of predilection such as the aortic root, aortic arch, common carotid, superior mesenteric, renal and pulmonary arteries. Sequentially, it is observed monocyte attachment to endothelial cells and a disruption of the subendothelial elastic lamina, lesions containing foam cells

and smooth muscle cells, and the development of fibrous plaques [2, 14, 16]. A Western-type diet is usually used to accelerate the development of these lesions in this mouse model. Hypercholesterolemia leads to endothelial dysfunction in both conductance and resistance vessels and to atherosclerotic lesions in conductance vessels of the apoE^{-/-} mouse, as recently reviewed by Meyrelles et al. [17].

Resting heart rate and blood pressure

In short-term direct and indirect hemodynamic measurements in conscious apoE^{-/-} mice, it has been shown that resting BP and HR (derived from pressure waves) are normal in young [3, 18-20] and increased in adult [21] animals, but other studies did not observe abnormal resting values of BP and HR in these animals even when they were fed a Western-type diet [22, 23]. Considering that BP fluctuates according to circadian rhythm and that continuous and prolonged recording of BP may result in better physiological values, an alternative method using a miniaturized radiotelemetry device seems to be the most suitable method for HR and BP measurements [24, 25]. This method allows for continuous, direct, and wireless recordings taken over months, for the assessment of short- and long-term BP and HR variability. Using this method, Pelat et al. [24] observed significantly higher mean 24-hour systolic BP (137 vs. 113 mmHg) and diastolic BP (100 vs. 89 mmHg) values and higher mean 24-hour HR (509 vs. 440 bpm) in apoE^{-/-} mice compared with wild-type control mice. The increased basal HR in the apoE^{-/-} mouse does not appear to be due to intrinsic changes in the discharge of the sinus node because ganglionic blockade has revealed a normal intrinsic HR in this animal [25]. Along with revealing tachycardia and hypertension in apoE^{-/-} mice, this method has enabled the observation of a total abolition of BP and HR circadian cycles [24]. This is an important finding because abnormal circadian variations of these parameters are known to be early signs of disease and are associated with an elevated risk of cardiovascular events, as reviewed elsewhere [26]. Therefore, as in humans, this mouse model shows that hypercholesterolemia and/or atherosclerosis are linked to important alterations in cardiac and hemodynamic parameters. This highlights the relevance of the apoE^{-/-} mouse as a model of dyslipidemia-related cardiovascular disorders. Next we highlight and discuss the involvement of the neurohumoral and autonomic nervous systems in the cardiovascular abnormalities of the apoE^{-/-} mouse model.

Fig. 1. Plasma lipid profile and temporal progression of atherosclerotic lesions in the apoE^{-/-} mouse. There is consensus that a Western-type diet and aging aggravate these dysfunctions, but the influence of gender is still controversial. Abbreviations: TG, triglycerides; PC, plasma cholesterol; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein. Modified from Meyrelles et al. [17], with permission.

Neurohumoral and autonomic nervous control of heart rate and blood pressure

Autonomic neural regulation of the circulation through the sympathetic and parasympathetic nervous systems provides optimal perfusion of organs in accordance with their metabolic needs. Activation of the sympathetic innervation of the heart and blood vessels causes vasoconstriction and increases in HR and cardiac contraction. In contrast, activation of the vagal innervation causes decreases in HR.

The activity of the cardiac and vascular autonomic nervous systems is the main determinant of resting BP and HR values and their circadian variations. Moreover, conditions in which sympathoexcitation (increasing HR, cardiac output and BP) is excessive and parasympathetic activation (slowing HR) is suppressed, as often occur in conjunction with several cardiovascular diseases states, increase the risks of morbidity and mortality (see Abboud [27] and Chappleau and Sabharwal [28]). The evaluation of the cardiac parasympathetic and sympathetic tones in the mouse has traditionally taken place through pharmacological methods involving a β -blocker (propranolol or atenolol), a muscarinic receptor blocker (atropine) or a ganglionic blocker (hexamethonium) [24, 25, 28, 29]. In the wild-type mouse under resting conditions, it is generally accepted that the cardiac sympathetic tone is predominant over the vagal tone [29, 30], but studies have provided convincing evidence that the cardiac vagal tone dominates the autonomic control of resting HR when housing conditions are taken into account [31]. Despite the fact that the apoE^{-/-} mouse was

created two decades ago, few studies have been proposed to evaluate its cardiac autonomic control of HR. One study has indicated that this animal model has defective parasympathetic drive to the heart [24], but another study indicated that this abnormality was observed only when the animals were fed an atherogenic diet [25]. It has been observed that the sympathetic tone is increased in apoE^{-/-} mice even when they are fed a normal chow diet, and this increase was further exacerbated in animals under a high-fat diet regimen [25].

An imbalance in the autonomic nervous system can also affect BP and HR variability, which may be associated with target organ damage and an increased risk of arrhythmias and sudden cardiac death [27]. Oscillations in BP and HR occur at different frequencies because of the influence of central autonomic rhythms, local vascular mechanisms, respiratory patterns, and circulating factors, as recently reviewed [32]. BP and HR variability in the mouse can be assessed by analyzing BP and HR recordings using spectral analysis with a fast Fourier transformation algorithm, which calculates different frequency bands [24, 28]. This method has revealed that in the mouse, cardiac vagal activity affects the variability of HR at high frequencies (1.5 to 5.0 Hz), in contrast with sympathetic nervous activity, which influences low-frequency (0.4 to 1.5 Hz) HR oscillations [32]. Using this method, Pelat et al. [24] observed that in apoE^{-/-} mice, HR and systolic BP variabilities increased in a very-low-frequency band (< 0.4 Hz), and HR variability decreased in the high-frequency band. These findings indicate an abnormal neurohumoral control of

Fig. 2. Schematic illustration describing hypothetical factors influencing dysautonomia in the apoE^{-/-} mouse. Abbreviations: BP, blood pressure; HF, high-frequency; HR, heart rate; RAS, renin-angiotensin system; ROS, reactive oxygen species; VLF, very-low-frequency.

HR and BP and defective parasympathetic influence on the heart, respectively, in apoE^{-/-} mice [24]. Although few studies have focused on dissecting neurohumoral and autonomic control of HR and BP in the apoE^{-/-} mouse [24, 25], there appears to be an association between hypercholesterolemia and/or atherosclerosis and cardiac autonomic dysfunction leading to changes in resting BP and HR, changes in BP and HR variabilities, and the abolition of BP and HR circadian rhythms. The LDL receptor knockout mouse, a model that needs to be fed with a hypercholesterolemic diet to reach similar high cholesterol levels spontaneously developed by the apoE^{-/-} mouse, has shown an increase in vascular sympathetic modulation and a decrease in cardiac parasympathetic modulation [33]. However, in contrast with the apoE^{-/-} mouse, the LDL receptor knockout mouse did not exhibit hypertension or tachycardia. In addition, parasympathetic activity observed in the high-frequency band was not altered [33]. Therefore, the apoE^{-/-} mouse is unique in terms of its cardiac and vascular phenotypes, and relevant data indicate an interplay among hypercholesterolemia/atherosclerosis and hypertension/tachycardia and HR/BP variability (see the illustration in Fig. 2), highlighting this mouse's importance as a model of dyslipidemia-associated cardiovascular diseases in humans.

Considering that the neuronal nitric oxide synthase (nNOS)-deficient mouse exhibits an increased resting HR

and decreased HR variability due primarily to loss of parasympathetic control [34], it is possible that NO produced in cardiac autonomic neurons plays a role in regulating HR in apoE^{-/-} mice. Indeed, treatment of ApoE^{-/-} mice with rosuvastatin resulted in a decrease in the expression of caveolin (an inhibitor of endothelial NOS), normalization of HR and BP variabilities, and restoration of chronotropic circadian cycles [24]. Although an interplay among cardiac dysautonomia, atherosclerosis and ROS has been postulated [27], this interaction has not been shown in the apoE^{-/-} mouse. The exaggerated and sustained sympathetic activity and reduced parasympathetic activity observed in the apoE^{-/-} mouse highlight the importance of this mouse model; in humans, reciprocal dysautonomia correlates strongly with increased mortality [27]. However, more experimental insight is necessary to understand the precise interactions between the imbalance in NOS/ROS production and the renin-angiotensin system, in addition to the influence of aging and diet on these parameters.

Neural reflex regulation of cardiovascular function

Neural reflex mechanisms are of major importance in regulating cardiovascular function. As illustrated in Figure 3, mechanosensitive nerve endings that innervate

Fig. 3. Schematic illustration of the major sensory signals, autonomic efferents, and target organs of the neural control of circulation. Top panel: baroreceptors located in the carotid sinuses and the aortic arch are mechanosensors of blood pressure (BP) fluctuations, and chemoreceptors located in the carotid bodies are chemosensors of hypoxia and acidosis. Adapted from Abboud [27], with permission. Bottom panel shows a schematic illustration of evaluation of the baroreflex sensitivity using vasoactive agents. In resting (basal) conditions, at each arterial systole, carotid sinus and aortic arch baroreceptors are activated and action potentials are generated and transmitted to the brain stem, where the signals are integrated and result in the maintenance of an inhibitory parasympathetic outflow to the heart and an excitatory sympathetic outflow to the heart and vessels. An acute phenylephrine-induced increase in BP leads to increase in the number of action potentials generated at each discharge and consequently to a reflex increase in the parasympathetic and decrease in the sympathetic nerve activities. The opposite is observed during an acute sodium nitroprusside-induced decrease in BP.

adventitia of the carotid sinuses and the aortic arch are specialized sensors of BP fluctuations (baroreceptors). In addition, chemosensitive nerve endings of carotid bodies sense hypoxia, hypercapnia, and/or acidosis (chemoreceptors). Afferent neural signals travel through the nodose and petrosal ganglia to the nucleus tractus solitarius in the medulla oblongata, promoting continuous circulatory and ventilator adjustments through the autonomic nervous system. As discussed below, the effects of these arterial sensors on efferent sympathetic nerve activity innervating blood vessels, heart, kidneys and other organs impacting cardiovascular function are opposing. The baroreceptor reflex is inhibitory, whereas the chemoreceptor reflex is excitatory (reviewed in [27, 28, 35-37]).

The arterial baroreceptor reflex (or baroreflex) system is one of the most powerful and rapidly acting mechanisms for controlling BP, providing moment-to-moment negative feedback regulation of BP and thereby

opposing large fluctuations or lability of BP and the adverse consequences of such fluctuations. In brief, an immediate increase in BP evokes a further reflex increase in cardiovagal activity and a decrease in cardiac and vascular sympathetic activity, resulting in a corresponding decrease in HR and correction of BP (see schematic illustration in Fig. 3, bottom panel). Conversely, in response to a decrease in BP, cardiovagal activity is inhibited and cardiac and vascular sympathetic activity increase, causing an increase in HR and an adjustment of BP. Activation of carotid chemoreceptors elicits an initial rise in BP that is mediated by an increase in sympathetic drive to the vasculature and bradycardia, which is produced by an increase in parasympathetic drive to the heart [38].

Studies in this area of research are important because alterations in the autonomic neural control of BP and HR are primary outcomes of dyslipidemia, hypertension and other cardiovascular diseases [27, 28, 37]. Our laboratory has demonstrated impaired baroreflex

Fig. 4. Schematic illustration of the protocol used for the assessment of baroreflex and chemoreflex function. Carotid occlusion rapidly reduces carotid sinus pressure (baroreceptor discharge decreases) and oxygen levels in the carotid body (chemoreceptor discharge increases), resulting in reflex-mediated increases in sympathetic nerve activity, vascular resistance and systemic blood pressure. Adenoviruses containing the gene reporter β -gal or the antioxidant superoxide dismutase (SOD) and catalase (CAT) genes were applied topically to the left carotid sinus in wild-type C57 mice and in $\text{apoE}^{-/-}$ mice, respectively, four days before observation. The increase in arterial blood pressure in response to sequential unilateral carotid artery occlusion followed by additional contralateral occlusion was impaired in anesthetized $\text{apoE}^{-/-}$ mice, but was normalized by SOD/CAT gene therapy, compared with wild-type mice. The results are based on data from Meyrelles and Chapleau published as an abstract [51].

function in mice with different pathophysiological conditions [26, 39-41]. The maintenance of arterial BP at adequate levels depends not on one system but on a series of interactions among sensing mechanisms such as baroreceptors and chemoreceptors capable of recognizing changes in parameters related to cardiovascular function [37]. As recently reviewed by Abboud [27], the impaired baroreflex function observed in several cardiovascular diseases is associated with enhanced chemoreceptor sensitivity, resulting in excessive sympathoexcitation. One of the objectives of the present review is to summarize the main experimental approaches being used to assess and to evaluate the neural reflex control of circulation in the $\text{apoE}^{-/-}$ mouse.

Assessment of baroreflex sensitivity in the mouse

Mouse models provide a powerful tool for the study of mechanisms underlying the dysfunction of autonomic reflex control of cardiovascular function as well as mechanisms that disrupt sensory signaling and result in or contribute to cardiovascular diseases [27, 35-37].

Baroreceptor dysfunction can lead to the disruption in the balance between parasympathetic and sympathetic tones [27]. Baroreflex sensitivity in the mouse can be evaluated by measuring changes in HR or in peripheral sympathetic nerve activity in response to drug-induced changes in BP. In brief, evaluation of cardiac baroreflex control of HR is performed by monitoring changes in resting BP induced pharmacologically by administering intravenously the vasodilator sodium nitroprusside and the vasoconstrictor phenylephrine and measuring the reflex tachycardia and bradycardia, respectively [26, 39]. However, vasoactive agents may affect autonomic centers, and repeated injections of fluid may limit the number of times the baroreflex can be evaluated in mice [32]. The bilateral carotid occlusion reflex, which rapidly reduces carotid sinus pressure and baroreceptor activity and results in baroreflex-mediated increases in sympathetic nerve activity, vascular resistance and systemic BP, lacks these limitations [35]. Our lab has also evaluated baroreceptor neurons and baroreflex function using different methods and incorporating viral-mediated gene transfer approaches that enable site-specific increases or decreases in gene expression [41-44]. An additional alternative is the analysis of spontaneous

Fig. 5. Angiotensin-mediated baroreflex dysfunction in the apoE^{-/-} mouse. Histological sections show immunohistochemistry of aortic arches from an 18-week-old apoE^{-/-} mouse compared with an age-matched C57 mouse by use of a polyclonal antibody against Ang II AT₁ receptors, counterstained with hematoxylin (scale bar: 10 μ m). Arrows indicate the presence of AT₁. Abbreviations: L, lumen; I, intima layer; M, media layer; A, adventitia layer; NI, neointima layer. The bar graph shows impaired baroreflex gain (sensitivity) in the apoE^{-/-} mice compared with C57 control mice ($p < 0.05$).

Fig. 6. Hypothetical scheme illustrating the role of ROS and decreased NO bioavailability in baroreflex and chemoreflex dysfunction under conditions of hypercholesterolemia and atherosclerosis. Several factors, including the RAS, ROS, aging and diet, may contribute to the impairment of baroreceptor function and the exacerbation of chemoreceptor function. Conversely, the impairment of arterial baroreceptors and the activation of chemoreceptors lead to a powerful additive sympathoexcitation. Abbreviations: ET, endothelin; IL, interleukin; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; $\cdot\text{O}_2^-$, superoxide anion; PARP, poly(ADP-ribose) polymerase; RAS, renin-angiotensin system; ROS, reactive oxygen species.

baroreceptor sensitivity using spontaneous fluctuations in BP and HR, which enables the measurement of baroreflex sensitivity during natural behaviors [45]. Therefore, slow fluctuations in BP and HR (e.g., circadian rhythms) can

be assessed in conscious, unrestrained mice through long-term recordings of these parameters [24, 46, 47], and short-term cardiovascular variability can be quantified by spectral analysis [24, 45, 47-49], which has revealed a

vagal predominance in the baroreflex control of HR in the mouse. Hans et al. [25] used the sequential method of analysis of HR and BP and observed that apoE^{-/-} mice fed a high-fat diet exhibit decreased spontaneous baroreceptor reflex sensitivity associated with reduced cardiovascular activity and increased sympathetic activity. However, as recently reviewed by Young and Davisson [32], the above techniques have their limitations, including the possibility of influence by respiration or circulating hormones and the fact that baroreceptor denervation does not completely eliminate parallel fluctuations in BP and HR, indicating that spontaneous oscillations in BP may not be caused exclusively by baroreflex-mediated mechanisms.

Baroreflex dysfunction: the role of ROS

Although examinations of baroreflex sensitivity in the apoE^{-/-} mouse have been conducted in few laboratories to date, several studies show that apoE^{-/-} mice exhibit impaired baroreflex function, which includes an imbalance in the sympathetic and parasympathetic control of HR and altered sympathetic nerve activity controlling BP and renal function [24, 32, 50, 51]. It is also known that the activation of baroreceptors, in addition to inhibiting sympathetic nerve activity, suppresses excitatory chemoreceptor activity. Consequently, impaired baroreflex function in this experimental model is often accompanied by enhanced chemoreceptor reflex activation [52], a state termed reciprocal dysautonomia [27]. Considering the potential clinical relevance of interactions between the chemoreflex and baroreflex, we suggest assessing both of these reflexes in experimental studies in murine models of cardiovascular pathophysiologies, and preferably assessing both in the same animal.

In cardiovascular diseases such as hypertension and hypercholesterolemia, a putative mechanism involved in baroreflex dysfunction and the imbalance of the parasympathetic (diminished) and sympathetic (increased) cardiac and vasomotor tones appears to be the generation of ROS in the components of the baroreflex and chemoreflex arches, as recently reviewed by Abboud [27].

The role of ROS in baroreflex function in apoE^{-/-} mice has been evaluated by our collaborators using viral-mediated gene transfer approaches [40-43]. In control mice, it has been shown that the carotid baroreflex is significantly impaired by the acute generation of oxygen radicals in the carotid sinus adventitia (xanthine) and is

abolished by carotid sinus denervation [43]. In young hypercholesterolemic apoE^{-/-} mice, an impaired carotid baroreflex was observed, and adenoviral-mediated gene transfer of superoxide dismutase (SOD) and catalase (CAT) to the carotid sinus adventitia restored the carotid occlusion reflex [51]. It is interesting that the authors observed that topical SOD/CAT gene transfer to one carotid sinus was able to restore bilateral baroreflex and chemoreflex function (see schematic illustration in Fig. 4) [51]. Further studies should investigate if the adenovirus expressing the SOD/CAT genes applied topically to the carotid sinus region can retrogradely reach central integrative areas and modulate the baroreflex and chemoreflex function. Therefore, gene transfer provides a complementary strategy to investigate the molecular mechanisms of cardiovascular regulation in genetically modified mice, and local injection of viral vectors enables site-specific increases in gene expression that can recover neural baroreflex function in knockout mice. These results indicate that ROS generated close to the nerve endings contribute to baroreflex dysfunction in this model of hypercholesterolemia and that gene transfer of antioxidant enzymes to the carotid sinus can restore baroreflex sensitivity.

In other studies, NADPH oxidase activity was found to be increased in the sympathetic ganglia of apoE^{-/-} mice, indicating that oxidative stress at this site may contribute to impaired baroreflex control of sympathetic nerve activity [53]. Sun et al. [54] showed an impaired carotid occlusion reflex in young (<15 weeks) apoE^{-/-} mice, but the reflex recovered with age (25-45 weeks) and was associated with increasing severity of atherosclerosis. The same authors showed that in 35-45-week-old apoE^{-/-} mice, the pressor response to bilateral carotid artery occlusion decreases during 100% oxygen ventilation but not during room air ventilation, suggesting a selective impairment of carotid baroreflex control of BP accompanied by enhanced chemoreceptor reflex activation [52]. Taking together, the decreased baroreflex sensitivity discussed above may reflect primarily defects in afferent/central signaling. Furthermore, these results and those previously discussed indicate that the generation of ROS at autonomic neurons significantly contributes to lipid-related cardiovascular diseases through a sympathoexcitation process.

Studies also support the concept that there is a link between ROS-induced oxidative damage to DNA in atherosclerosis and overexpression of poly(ADP-ribose) polymerase (PARP) [25, 55]. In agreement, Hans et al. [25] showed a significant reduction in spontaneous

baroreflex sensitivity in ~20-week-old male apoE^{-/-} mice fed regular or high-fat diets, and these researchers also showed that PARP-1 gene deletion restored baroreflex sensitivity in apoE^{-/-} mice when they were fed a regular diet but not when fed a high-fat diet. These findings suggest that PARP-1 gene deletion may improve baroreflex sensitivity at early stages of atherosclerosis and that a high-fat diet reverses the beneficial effects of PARP-1 deletion on baroreflex function.

The above-mentioned findings can be seen as evidence that the enhanced inhibitory influence of ROS in baroreceptor neurons impairs the baroreceptor reflex and contributes to dysautonomia in apoE^{-/-} mice. This condition is worsened by the reduction in antioxidant gene expression that accompanies aging and by a Western-type diet. Based on these findings, we expect experimental studies to show a restoration of baroreflex sensitivity and parasympathetic tone and a reduction in sympathoexcitation with antioxidant therapies in the apoE^{-/-} mouse.

Baroreflex dysfunction: the role of the renin-angiotensin system

The renin-angiotensin system also appears to contribute to baroreflex dysfunction. Apart from its pressure effect, Ang II acts at AT₁ receptors to decrease the sensitivity of aortic afferents during physiological changes in BP [56]. In the presence of hypercholesterolemia, Ang II production is augmented mainly by chymase, which is released by mast cells localized at the adventitia layer [57, 58], in contrast with the angiotensin-converting enzyme-dependent Ang II, which is distributed through all vessel layers in C57 mice. Figure 5 shows data from our laboratory that demonstrate the typical immunoreactivity of Ang II AT₁ receptors at the site of baroreceptor endings at the adventitia layer of the aortic arch of an 18-week-old apoE^{-/-} mouse, contrasted with an age-matched C57 mouse showing a uniform distribution of AT₁ receptors through all vessel layers. We also evaluated the baroreflex sensitivity in these animals, in conscious conditions, and observed a decreased baroreflex gain in apoE^{-/-} mice compared with C57 animals (Fig. 5, bar graph). Taken together these findings suggest that hypercholesterolemia-induced increases in adventitial Ang II and its AT₁ receptors at the aortic arch (and probably at the carotid sinus) contribute to the impairment of the baroreflex function in apoE^{-/-} mice. In accordance, inter-breeding apoE^{-/-} and

renin-angiotensinogen transgenic mice yields mice with an impaired baroreflex at a young age prior to heart failure and the impairment persists during heart failure despite the reversal of hypertension [59].

Baro- and chemoreflex dysfunction: new insights

Recently, it has been investigated acid-sensing ion channels (ASICs) as molecular components of sensory signals at arterial mechano- and chemoreceptors, including studies with an ASIC knockout mouse [60]. They have shown that ASIC subfamily members are important to baroreceptor mechanotransduction (ASIC2) and the transduction of acid sensitivity by chemoreceptors (ASIC3). One might ask how ASIC can be important to neuroreflex function; the answer lies in the fact that the ASIC knockout mouse shows an increased chemoreceptor component and a decreased baroreceptor component of the bilateral carotid occlusion pressor response (see a scheme of the method in Fig. 4) [61, 62]. In addition, it has been shown that treatment of ASIC2 knockout mice with a mimetic of superoxide dismutase, tempol, a superoxide anion scavenger, restores baroreflex sensitivity, reduces BP and sympathoexcitation, and restores the parasympathetic tone [61], features that have also been observed in the apoE^{-/-} mouse as discussed in previous sections. Therefore, as in the ASIC2 knockout model, we postulated that there is an important inhibitory influence of ROS on the impairment of the baroreflex and in the dysautonomia observed in the apoE^{-/-} mouse. Further studies using this model are needed and are expected to aid in defining lipid-related cardiovascular pathophysiology at the molecular level.

Figure 6 summarizes the interplay among the NO/ROS imbalance, local and systemic Ang II, the type of diet and aging, and the contributions of these factors to the impairment of the neural control of HR and BP in the apoE^{-/-} mouse. Future studies should provide additional insights into the mechanisms by which the neuroreflex becomes impaired in this model of hypercholesterolemia and atherosclerosis.

For the interested reader, a number of other reviews provide information on the assessment of neurocardiovascular regulation in the wild-type mouse [32] and on lipid profiles and atherosclerotic lesions [63-65], endothelial dysfunction [17], cardiac and vascular phenotypes [66-68], diet [69] and pharmaceutical modifiers [70] in the apoE^{-/-} mouse model.

Conclusions

Despite some discrepancies, long-term recordings of HR and BP using telemetry devices show convincing evidence that apoE^{-/-} mice exhibit tachycardia and hypertension accompanied by abnormal neurohumoral and autonomic control of cardiovascular function. This dysfunction is characterized by decreased cardiovagal activity and increased sympathetic activity, abnormal HR and BP variabilities and abolition of the circadian cycles of HR and BP, primarily under the influence of aging and a Western-type diet. Moreover, the renin-angiotensin system appears to play a dual role, contributing additively with ROS to DNA damage and further with aging to dysautonomia in this animal model. Studies also provide clear evidence that in this mouse model, baroreflex control of HR and BP, as well as of the chemoreflex, are altered and are associated with multiple modulating factors including the generation of ROS and hypercholesterolemia-induced increases of adventitial Ang II. The importance of these phenotypes, i.e., dysautonomia and impaired reflex control of HR and BP, in this mouse model is highlighted by the fact that the reciprocal changes in

baro- and chemoreflex promote sympathovagal imbalance and increase mortality in humans [27]. Furthermore, the apoE^{-/-} mouse model, in addition to contributing to the understanding of the etiology of hypercholesterolemia and atherosclerosis in humans, provides a powerful approach for investigation of the underlying mechanisms in the neurohumoral and autonomic control of lipid-related cardiovascular dysfunctions.

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References

- 1 Borst O, Ochmann C, Schönberger T, Jacoby C, Stellos K, Seizer P, Flögel U, Lang F, Gawaz M: Methods employed for induction and analysis of experimental myocardial infarction in mice. *Cell Physiol Biochem* 2011;28:1-12.
- 2 Pereira TM, Nogueira BV, Lima LC, Porto ML, Arruda JA, Vasquez EC, Meyrelles SS: Cardiac and vascular changes in elderly atherosclerotic mice: the influence of gender. *Lipids Health Dis* 2010;9:87.
- 3 Arruda RM, Peotta VA, Meyrelles SS, Vasquez EC: Evaluation of vascular function in apolipoprotein E knockout mice with angiotensin-dependent renovascular hypertension. *Hypertension* 2005;46:932-936.
- 4 Cola MS, Gava AL, Meyrelles SS, Vasquez EC: Endothelial dysfunction of resistance vessels in female apolipoprotein E-deficient mice. *Lipids Health Dis* 2010;9:51.
- 5 Balarini CM, Oliveira MZ, Pereira TM, Silva NF, Vasquez EC, Meyrelles SS, Gava AL: Hypercholesterolemia promotes early renal dysfunction in apolipoprotein E-deficient mice. *Lipids Health Dis* 2011;10:220.
- 6 Zhang SH, Reddick RL, Piedrahita JA, Maeda N: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 1992;258:468-471.
- 7 Plump AS, Smith JD, Hayek T, Aalto-Setälä K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL: Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 1992;71:343-353.
- 8 Piedrahita JA, Zhang SH, Hageman JR, Oliver PM, Maeda N: Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc Natl Acad Sci USA* 1992;89:4471-4475.
- 9 Zhang SH, Reddick RL, Burkey B, Maeda N: Diet-induced atherosclerosis in mice heterozygous and homozygous for apolipoprotein E gene disruption. *J Clin Invest* 1994;94:937-945.
- 10 Bourassa PA, Milos PM, Gaynor BJ, Breslow JL, Aiello RJ: Estrogen reduces atherosclerotic lesion development in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA* 1996;93:10022-10027.
- 11 Bonthou S, Heistad DD, Chappell DA, Lamping KG, Faraci FM: Atherosclerosis, vascular remodeling, and impairment of endothelium-dependent relaxation in genetically altered hyperlipidemic mice. *Arterioscler Thromb Vasc Biol* 1997;17:2333-2340.
- 12 d'Uscio LV, Barton M, Shaw S, Lüscher TF: Chronic ET_A receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. *Cardiovasc Res* 2002;53:487-495.

- 13 Surra JC, Guillén N, Arbonés-Mainar JM, Barranquero C, Navarro MA, Arnal C, Orman I, Segovia JC, Osada J: Sex as a profound modifier of atherosclerotic lesion development in apolipoprotein E-deficient mice with different genetic backgrounds. *J Atheroscler Thromb* 2010;17:712-721.
- 14 Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R: ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb* 1994;14:133-140.
- 15 Jiang F, Gibson AP, Dusting GJ: Endothelial dysfunction induced by oxidized low-density lipoproteins in isolated mouse aorta: a comparison with apolipoprotein-E deficient mice. *Eur J Pharmacol* 2001;424:141-149.
- 16 Yamashita T, Kawashima S, Ozaki M, Namiki M, Shinohara M, Inoue N, Hirata K, Umetani K, Yokoyama M: In vivo angiographic detection of vascular lesions in apolipoprotein E-knockout mice using a synchrotron radiation microangiography system. *Circ J* 2002;66:1057-1059.
- 17 Meyrelles SS, Peotta VA, Pereira TM, Vasquez EC: Endothelial dysfunction in the apolipoprotein E-deficient mouse: insights into the influence of diet, gender and aging. *Lipids Health Dis* 2011;10:211.
- 18 Wassmann S, Czech T, van Eickels M, Fleming I, Böhm M, Nickenig G: Inhibition of diet-induced atherosclerosis and endothelial dysfunction in apolipoprotein E/angiotensin II type 1A receptor double-knockout mice. *Circulation* 2004;110:3062-3067.
- 19 Nogueira BV, Peotta VA, Meyrelles SS, Vasquez EC: Evaluation of aortic remodeling in apolipoprotein E-deficient mice and renovascular hypertensive mice. *Arch Med Res* 2007;38:816-821.
- 20 Stapleton PA, Goodwill AG, James ME, Frisbee JC: Altered mechanisms of endothelium-dependent dilation in skeletal muscle arterioles with genetic hypercholesterolemia. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R1110-1119.
- 21 Yang R, Powell-Braxton L, Ogaoawara AK, Dybdal N, Bunting S, Ohneda O, Jin H: Hypertension and endothelial dysfunction in apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol* 1999;19:2762-2768.
- 22 Barton M, Haudenschild CC, d'Uscio LV, Shaw S, Münter K, Lüscher TF: Endothelin ET_A receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci U S A* 1998;95:14367-14372.
- 23 Custodis F, Baumhäkel M, Schlimmer N, List F, Gensch C, Böhm M, Laufs U: Heart rate reduction by ivabradine reduces oxidative stress, improves endothelial function, and prevents atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2008;117:2377-2387.
- 24 Pelat M, Dessy C, Massion P, Desager JP, Feron O, Balligand JL: Rosuvastatin decreases caveolin-1 and improves nitric oxide-dependent heart rate and blood pressure variability in apolipoprotein E^{-/-} mice in vivo. *Circulation* 2003;107:2480-2486.
- 25 Hans CP, Feng Y, Naura AS, Zerfaoui M, Rezk BM, Xia H, Kaye AD, Matrougui K, Lazartigues E, Boulares AH: Protective effects of PARP-1 knockout on dyslipidemia-induced autonomic and vascular dysfunction in ApoE mice: effects on eNOS and oxidative stress. *PLoS One* 2009;4:e7430.
- 26 Agarwal R: Regulation of circadian blood pressure: from mice to astronauts. *Curr Opin Nephrol Hypertens* 2010;19:51-58.
- 27 Abboud FM: The Walter B: Cannon Memorial Award Lecture, 2009. Physiology in perspective: The wisdom of the body. In search of autonomic balance: the good, the bad, and the ugly. *Am J Physiol Regul Integr Comp Physiol* 2010;298:R1449-1467.
- 28 Chapleau MW, Sabharwal R: Methods of assessing vagus nerve activity and reflexes. *Heart Fail Rev* 2011;16:109-127.
- 29 Peotta VA, Gava AL, Vasquez EC, Meyrelles SS: Evaluation of baroreflex control of heart rate in renovascular hypertensive mice. *Can J Physiol Pharmacol* 2007;85:761-766.
- 30 Janssen BJ, Smits JF: Autonomic control of blood pressure in mice: basic physiology and effects of genetic modification. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R1545-1564.
- 31 Swoap SJ, Li C, Wess J, Parsons AD, Williams TD, Overton JM: Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. *Am J Physiol Heart Circ Physiol* 2008;294:H1581-1588.
- 32 Young CN, Davisson RL: In vivo assessment of neurocardiovascular regulation in the mouse: principles, progress, and prospects. *Am J Physiol Heart Circ Physiol* 2011;301:H654-662.
- 33 Evangelho JS, Casali KR, Campos C, De Angelis K, Veiga ABG, Rigatto K: Hypercholesterolemia magnitude increases sympathetic modulation and coagulation in LDLr knockout mice. *Auton Neurosci* 2011;159:98-103.
- 34 Jumrussirikul P, Dinerman J, Dawson TM, Dawson VL, Ekelund U, Georgakopoulos D, Schramm LP, Calkins H, Snyder SH, Hare JM, Berger RD: Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice. *J Clin Invest* 1998;102:1279-1285.
- 35 Chapleau MW, Li Z, Meyrelles SS, Ma X, Abboud FM: Mechanisms determining sensitivity of baroreceptor afferents in health and disease. *Ann N Y Acad Sci* 2001;940:1-19.
- 36 Ma X, Abboud FM, Chapleau MW: Neurocardiovascular regulation in mice: experimental approaches and novel findings. *Clin Exp Pharmacol Physiol* 2003;30:885-893.
- 37 Vasquez EC, Meyrelles SS, Mauad H, Cabral AM: Neural reflex regulation of arterial pressure in pathophysiological conditions: interplay among the baroreflex, the cardiopulmonary reflexes and the chemoreflex. *Braz J Med Biol Res* 1997;30:521-532.
- 38 Braga VA, Burnmeister MA, Sharma RV, Davisson RL: Cardiovascular responses to peripheral chemoreflex activation and comparison of different methods to evaluate baroreflex gain in conscious mice using telemetry. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R1168-1174.
- 39 Peotta VA, Vasquez EC, Meyrelles SS: Cardiovascular neural reflexes in L-NAME-induced hypertension in mice. *Hypertension* 2001;38:555-559.
- 40 Gava AL, Peotta VA, Cabral AM, Meyrelles SS, Vasquez EC: Decreased baroreflex sensitivity in isoproterenol-treated mice with cardiac hypertrophy. *Auton Neurosci* 2004;114:47-54.
- 41 Gava AL, Peotta VA, Cabral AM, Vasquez EC, Meyrelles SS: Overexpression of eNOS prevents the development of renovascular hypertension in mice. *Can J Physiol Pharmacol* 2008;86:458-464.
- 42 Meyrelles SS, Mao HZ, Heistad DD, Chapleau MW: Gene transfer to carotid sinus in vivo: a novel approach to investigation of baroreceptors. *Hypertension* 1997;30:708-713.
- 43 Meyrelles SS, Sharma RV, Whiteis CA, Davidson BL, Chapleau MW: Adenovirus-mediated gene transfer to cultured nodose sensory neurons. *Brain Res Mol Brain Res* 1997;51:33-41.
- 44 Meyrelles SS, Chapleau MW: Analysis of carotid baroreflex using sequential carotid occlusion and gene transfer in mice. *Hypertension* 1999;33:A1085.
- 45 Oosting J, Struijker-Boudier HA, Janssen BJ: Validation of a continuous baroreceptor reflex sensitivity index calculated from spontaneous fluctuations of blood pressure and pulse interval in rats. *J Hypertens* 1997;15:391-399.

- 46 Janssen BJ, Leenders PJ, Smits JF: Short-term and long-term blood pressure and heart rate variability in the mouse. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R215-225.
- 47 Butz GM, Davisson RL: Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. *Physiol Genomics* 2001;5:89-97.
- 48 Gehrmann J, Hammer PE, Maguire CT, Wakimoto H, Triedman JK, Berul CI: Phenotypic screening for heart rate variability in the mouse. *Am J Physiol Heart Circ Physiol* 2000;279:H733-740.
- 49 Just A, Faulhaber J, Ehmke H: Autonomic cardiovascular control in conscious mice. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R2214-2221.
- 50 Ma XY, Abboud FM, Chapleau MW: Altered baroreflex control of renal sympathetic nerve activity in normotensive apo-E deficient atherosclerotic mice. *The Physiologist* 2000;43:A275.
- 51 Meyrelles SS, Chapleau MW: Impaired carotid occlusion reflex in apoE deficient mice and its reversal by gene transfer to carotid sinus. *J Hypertens* 2000;18(Suppl. 4):S202.
- 52 Sun W, Abboud FM, Chapleau MW: Altered baro- and chemoreflex sensitivity revealed by carotid artery occlusion reflex in apo-E knockout mice. *FASEB J* 2001;15:A1146.
- 53 Ma X, Zhang HJ, Whiteis CA, Tian X, Davisson RL, Kregel KC, Abboud FM, Chapleau MW: NAD(P)H oxidase-induced oxidative stress in sympathetic ganglia of apolipoprotein E deficient mice. *Auton Neurosci* 2006;126-127:285-291.
- 54 Sun W, Abboud FM, Chapleau MW: Impaired carotid occlusion reflex in Apo-E deficient mice is normalized with age and increasing severity of atherosclerotic lesions. *The Physiologist* 2000;43:A276.
- 55 Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM: Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation* 2002;106:927-932.
- 56 Santos CM, Moreira ED, Krieger EM, Michelini LC: Chronic AT1 receptor blockade alters aortic nerve activity in hypertension. *Hypertension* 1998;31:973-977.
- 57 Arakawa K, Urata H: Hypothesis regarding the pathophysiological role of alternative pathways of angiotensin II formation in atherosclerosis. *Hypertension* 2000;36:638-641.
- 58 Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA: Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT_{1A} receptor. *Circulation* 2004;110:3849-3857.
- 59 Sabharwal R, Weiss RM, Stauss HM, Sigmund CD, Abboud FM, Chapleau MW: ApoE^{-/-}R^{+/+} mice with hypercholesterolemia, hypertension, and decreased baroreflex sensitivity spontaneously develop heart failure with age. *FASEB J* 2007;21:753.14.
- 60 Lu Y, Ma X, Sabharwal R, Snitsarev V, Morgan D, Rahmouni K, Drummond HA, Whiteis CA, Costa V, Price M, Benson C, Welsh MJ, Chapleau MW, Abboud FM: The ion channel ASIC2 is required for baroreceptor and autonomic control of the circulation. *Neuron* 2009;64:885-897.
- 61 Sabharwal R, Chapleau MW, Price MP, Welsh MJ, Abboud FM: Molecular mechanisms of baro- and chemoreceptor activation: evidence that ASIC1 and ASIC3 contribute to chemoreceptor activation (Abstract). *Circulation* 2005;112:156.
- 62 Sabharwal R, Stauss HM, Lazartigues E, Whiteis CA, Price MP, Welsh MJ, Davisson RL, Abboud FM, Chapleau MW: ASIC2^{-/-} mice: a genetic model of impaired baroreceptor activation and oxidative stress-dependent neurogenic hypertension (Abstract). *Hypertension* 2006;48:e31.
- 63 Hofker MH, van Vlijmen BJM, Havekes LM: Transgenic mouse models to study the role of APOE in hyperlipidemia and atherosclerosis. *Atherosclerosis* 1998;137:1-11.
- 64 Jawien J, Nastalek P, Korbut R: Mouse models of experimental atherosclerosis. *J Physiol Pharmacol* 2004;55:503-517.
- 65 Meir KS, Leitersdorf E: Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress. *Arterioscler Thromb Vasc Biol* 2004;24:1006-1014.
- 66 Wang Y-X: Cardiovascular functional phenotypes and pharmacological responses in apolipoprotein E deficient mice. *Neurobiol Aging* 2005;26:309-316.
- 67 Lu H, Cassis LA, Daugherty A: Atherosclerosis and arterial blood pressure in mice. *Curr Drug Targets* 2007;8:1181-1189.
- 68 Vasquez EC, Peotta VA, Gava AG, Pereira TMC, Meyrelles SS: Cardiac and vascular phenotypes in the apolipoprotein E-deficient mouse. *J Biomed Sci* 2012;19:22.
- 69 Imaizumi K: Diet and atherosclerosis in apolipoprotein E-deficient mice. *Biosci Biotechnol Biochem* 2011;75:1023-1035.
- 70 Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM, Kooistra T: Mouse models for atherosclerosis and pharmaceutical modifiers. *Arterioscler Thromb Vasc Biol* 2007;27:1706-1721.