Cardiovascular Neural Reflexes in L-NAME–Induced Hypertension in Mice

Veronica A. Peotta, Elisardo C. Vasquez, Silvana S. Meyrelles

Abstract—The mouse is the most used animal for studying the genetic basis of cardiovascular diseases. However, the mechanisms of regulation of cardiovascular function in this animal are not yet well understood. The goal of this study was to evaluate the baroreflex, the Bezold-Jarisch cardiopulmonary reflex (BJR), and the chemoreflex in mice with hypertension induced by inhibition of NO using Nω-nitro-L-arginine-methyl ester (L-NAME). Basal mean arterial pressure (MAP) measured under anesthesia (urethane, 1 mg/g IP) was significantly higher in L-NAME (400 μg/g IP for 7 days)–treated (HT) mice (n = 7) compared with vehicle-treated (NT; n = 10) animals (126±9 versus 79±2 mm Hg) without differences in heart rate (HR). Baroreflex sensitivity, evaluated using phenylephrine (1 μg/g IV) was enhanced in HT mice compared with NT mice (−9.8±1.4 versus −4.9±0.5 bpm/mm Hg). The BJR, induced by phenylbiguanide (40 ng/g IV), was significantly attenuated in HT animals (MAP, −13±5%; HR, −39±6%) compared with NT animals (MAP, −38±5%; HR, −66±2%). The chemoreflex, induced by potassium cyanide (0.26 μg/g IV), was significantly attenuated in HT animals (MAP, +14±4%; HR, −8±2%) compared with NT animals (MAP, +29±4%; HR, −15±4%). As has been observed in rats, chronic inhibition of NO synthase in mice results in arterial hypertension. Enhancement of baroreflex sensitivity and attenuation of BJR and chemoreflex seem to be mainly caused by inhibition of NO synthase because individual analyses did not show positive correlation between changes in these reflexes and MAP levels in the HT group. (Hypertension. 2001;38[part 2]:555-559.)

Key Words: hypertension, experimental ■ mice ■ L-NAME ■ baroreflex ■ reflex

Molecular biology techniques have permitted the development of genetically altered animals, in which genes controlling a specific function are overexpressed or disrupted to enable study of the contribution of the respective gene product to a specific disease. For practical reasons, the mouse has been the most genetically altered animal. Although transgenic mice are increasingly available to be used in cardiovascular research, knowledge about the normal physiological and pathophysiological characteristics of the murine cardiovascular system is still limited.

After the first demonstration that chronic administration of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) causes arterial hypertension in rats, and our demonstration that the sympathetic nervous system plays a major role in L-NAME–induced hypertension in rats, many interesting data have been obtained in this model of experimental hypertension. It has also been shown that L-NAME hypertension in rats is characterized by changes in baroreflex control of cardiovascular function, and a profound enhancement of the Bezold-Jarisch cardiopulmonary reflex. Although it has been demonstrated that chronic administration of L-NAME also leads to arterial hypertension in mice, no previous studies have systematically evaluated the effects of chronic inhibition of NO synthase on the neural reflex control of cardiovascular function in mice. Therefore, the purpose of this study was to evaluate the effects of chronic administration of L-NAME on arterial baroreflex, Bezold-Jarisch cardiopulmonary reflex, and chemoreflex in mice.

Methods

Animals

Experiments were conducted with male adult Swiss Webster mice (Physiological Sciences Animal Care) weighing 28 to 34 g. The animals were caged individually and had free access to food and water. Room temperature was maintained at 23±1°C, and a 12-hour/12-hour light/dark cycle was in effect. Experiments were conducted in accordance with the guidelines for the care and use of animals (Federation of Societies of Experimental Biology [FeSEB]) and approved by the local committee.

Technique

The animals were treated with L-NAME (400 μg/g IP daily, Sigma Chemical Co) or given vehicle (saline, 13 μL/g IP daily) for 7 days. At the end of treatment, the animals were anesthetized with urethane (1 mg/g IP), which is known to maintain a great portion of the cardiovascular reflexes, and supplemental anesthesia was administered as needed. Because mice are susceptible to hypothermia, especially when anesthetized, they were placed on a warming pad.
(averaging 38°C) throughout the surgical procedure and experimental protocols. Sterile heparinized saline-filled (50 U/mL) catheters (Microrenathane; Braintree Science Inc), 0.1 cm OD \( \times \) 0.06 cm ID, drawn over heat were inserted with aid of a dissecting microscopy (Opto Eletronica SA) into the carotid artery and femoral vein. The arterial catheter was used for direct measurement of pulsatile and mean arterial pressure (MAP) and heart rate (HR) (by a cardiometer) by a polygraph (Gould), and the venous catheter was used for administration of drugs. To avoid or minimize a possible influence of each drug on the subsequent test and based on our preliminary observation, a recovery period of \( \sim \)15 minutes was allowed for arterial pressure and HR to return to baseline values before administering the next drug.

Arterial baroreflex control of HR was determined in anesthetized mice by producing acute changes in arterial pressure with infusion (10 \( \mu \)L IV for 10 seconds) of phenylephrine (1 \( \mu \)g/g, Sigma) and sodium nitroprusside (1 \( \mu \)g/g, Sigma) using an infusion pump (Harvard Apparatus Inc). To assess the Bezold-Jarisch reflex, a bolus injection of the 5-HT3-receptor agonist phenylbiguanide (40 ng/g IV, Sigma) was administered, and the simultaneous short-lasting fall of arterial pressure and bradycardia were recorded. The chemoreflex was evaluated by measuring the increase in arterial pressure and the nonreflex bradycardia following a bolus injection of potassium cyanide (0.26 \( \mu \)g/g IV, Sigma).

At the end of experiments, the hearts were removed, and wet heart weight was used for evaluation of cardiac hypertrophy by measuring the ratio of heart weight to body weight.

Data Analysis
Results are reported as mean\( \pm \)SEM. A 2-way ANOVA for repeated measurements was used for multiple comparisons, and the Student’s \( t \) test was used for comparison of 2 independent samples. The level of significance was taken as \( P<0.05 \).

Results
As expected and observed in Figure 1, mice treated with L-NAME for 7 days showed an expected arterial hypertension (126\( \pm \)7 mm Hg, \( P<0.01 \)) compared with that of control mice (79\( \pm \)2 mm Hg), without significant differences in basal HR (703\( \pm \)15 versus 676\( \pm \)21 bpm, respectively). Figure 1 (bottom panels) shows that L-NAME caused an immediate and significant reduction of body weight, which was maintained throughout the treatment. No significant differences in heart weight–to–body weight ratio were observed between L-NAME and control groups.

Figure 2 illustrates the reflex chronotropic responses to a phenylephrine-induced increase in arterial pressure of an L-NAME-treated mouse compared with a control mouse. As summarized in Figure 3 (left panels), although the phenylephrine-induced pressor responses were significantly attenuated in L-NAME-treated mice (+33\( \pm \)6\%, \( P<0.01 \), \( n=7 \)) compared with control mice (+97\( \pm \)5\%, \( n=10 \)), the reflex bradycardia was similar in both groups (-51\( \pm \)8\% versus -56\( \pm \)6\%), resulting in an increased baroreflex sensitivity in L-NAME-treated mice (-9.8\( \pm \)1.4 bpm/mm Hg, \( P<0.01 \)) compared with control mice (-4.9\( \pm \)0.5 bpm/mm Hg, \( P<0.01 \)). As shown in Figure 3 (right panels), the nitroprusside-induced hypotension was increased in L-NAME–treated mice compared with control mice, but no reflex tachycardia was observed in both groups, probably because of the high resting HR values.

Top panels of Figure 4 show typical recordings of phenylbiguanide-induced hypotension and bradycardia, which characterizes the Bezold-Jarisch reflex, demonstrating a smaller response in both duration and magnitude in the L-NAME–treated mice compared with the control mice. Figure 4 (bottom panels) summarizes the values of the Bezold-Jarisch reflex testing, showing a significant reduction
of the hypotension and bradycardia in L-NAME–treated mice (−14 ± 5 mm Hg and −274 ± 44 bpm, P < 0.01, n = 7) compared with control mice (−31 ± 5 mm Hg and −461 ± 26 bpm, n = 10).

The chemoreflex test showed significantly decreased presor and chronotrope responses in L-NAME–treated mice (MAP, +14 ± 4%; HR, −8 ± 2%; n = 6) compared with vehicle-treated mice (MAP, +29 ± 4%; HR, −15 ± 4%; n = 7).

Discussion

Studies into mechanisms underlying physiology and pathophysiological states have increasingly employed transgenic mouse models, in which targeted genes are functionally altered or deleted using molecular biology technologies.8–11 Concomitant to this process and despite its small size, the mouse is becoming an important species in cardiovascular research and could help us elucidate physiological states have increasingly employed transgenic and knock-out mouse models, in which targeted genes are functionally altered or deleted using molecular biology technologies.8–11 Concomitant to this process and despite its small size, the mouse is becoming an important species in cardiovascular research and could help us elucidate

of vehicle-treated mice without conflicting with the standards of the local animal care and use committee. Although mice are quite active, further studies are required to evaluate the mechanisms of cardiovascular control in conscious animals and in other murine models of experimental hypertension.

The present results are in good agreement with other reports showing that chronic administration of L-NAME causes arterial hypertension in mice1–3 and rats1 without significant changes in heart weight and heart rate. To achieve a high blood pressure value and a possible cardiac hypertrophy in the treated animals, we used a nontoxic high dose of L-NAME13 because the arterial pressure levels are dependent on the dose of L-NAME.7,13 Although the heart weight–to–body weight ratio (Figure 1, bottom panels) could indicate a tendency to cardiac hypertrophy in L-NAME–treated mice, this cannot be considered because there were no significant differences in heart weight between the 2 groups and because L-NAME–treated mice showed a reduced body weight throughout the treatment. The decrease in body weight in this group may be mainly caused by L-NAME treatment because NO seems to be involved in the regulation of appetite in mice.14

During the baroreflex testing and consistent with observations in rats,3–5 L-NAME–treated mice showed an attenuated pressor response to phenylephrine because, at least in part, the basal values of arterial pressure were already elevated. In contrast, it is conceivable that the exaggerated nitroprusside–induced hypotension in L-NAME mice could be caused by both the high basal arterial pressure levels and the direct donation of NO to the vessels. This observation indicates that the vascular relaxing components are functionally preserved. We found an enhanced sensitivity of the baroreflex against acute increases in arterial pressure, which could indicate that this neural reflex is upregulated in L-NAME–treated mice the same as has been observed in rats presenting L-NAME hypertension and high cardiac sympathetic tones.3 We speculate that the lack of reflex tachycardia during nitroprusside–induced falls in arterial pressure in both L-NAME–treated and vehicle-treated mice could be because of a high cardiac sympathetic tones as indicated by the high basal HR values in both groups. This hypothesis is based on another report15 showing that HR in Swiss mouse is predominantly under sympathetic control and that atropine has minor effects on cardiac rate. The most used method to evaluate the baroreflex sensitivity in other species has been through several randomly assigned doses of phenylephrine and sodium nitroprusside. However, considering the small size of the mouse and trying to avoid volume overloading, we opted for an infusion method that has been used in other studies with human beings16 and rats17 that required short-term tests. The development of more suitable approaches will be helpful to evaluate in a better manner the baroreflex function in the mouse.

Because it is well known that the cardiopulmonary reflexes contribute to the cardiovascular control by providing neurohumoral drive to the cardiovascular system and by interacting with arterial baroreflex,18–20 the Bezold-Jarisch reflex was also evaluated in this model of hypertension. We observed that both the magnitude and duration of the phenylebiguanide-
induced response was decreased in L-NAME–treated mice, but our data do not allow us to speculate in which part of the neural reflex arch it is located. Consistent with the observations of an enhanced baroreflex in L-NAME hypertensive mice, it is not entirely surprising that we have found in this study an attenuation of the Bezold-Jarisch reflex, perhaps as an inhibitory compensation. This result is opposite to our previous observations in L-NAME hypertensive rats that showed that this cardiopulmonary reflex was profoundly enhanced. Our data do not allow us to speculate if this discrepancy between species is due to different hemodynamic responses to NO synthase blockade. However, these differences could not be attributed to a high sympathetic drive to the heart and consequently to a high HR alone because the reflex bradycardia to phenylephrine during the baroreflex testing was enhanced in L-NAME–treated mice. Both the enhanced baroreflex and the attenuated Bezold-Jarisch reflex do not appear to be secondary to arterial hypertension because individual analyses did not show positive correlations between changes in these reflexes and arterial blood pressure levels in L-NAME–treated mice. Thus, this could be mainly caused by inhibition of NO synthesis.

The chemoreflex was also significantly attenuated in L-NAME–treated mice. This finding corroborates the hypothesis that an enhanced buffering function of the arterial baroreflex in this hypertension model leads to a hyporesponsiveness of the cardiopulmonary reflexes and chemoreflex. In summary, we report that the chronic hypertensive effect of L-NAME is accompanied by an augmented sensitivity of the arterial baroreceptor control of HR to acute increases of arterial pressure and a significant attenuation of the Bezold-Jarisch reflex and the chemoreflex in mice. Although we can rule out the possibility of these changes be related with the arterial hypertension, the mechanisms by which the inhibition of the NO synthase mediates this changes remain unknown.

Acknowledgments

This study was supported by National Council for the Development of Science and Technology (CNPq), National Agency for Studies.

Figure 3. Graphs show average relative changes in MAP and HR in response to intravenous infusion of phenylephrine (1 μg/g body weight for 10 seconds; left panels) and sodium nitroprusside (1 μg/g body weight for 10 seconds; right panels) and the calculated maximum baroreflex sensitivity comparing L-NAME–treated (n=7) with control (n=10) mice. Values are mean±SEM. **P<0.01 vs control group.
and Projects (Finep), and Funds for Science and Technology of the City of Vitoria (Facitec-Vitoria).

References


Cardiovascular Neural Reflexes in L-NAME–Induced Hypertension in Mice
Veronica A. Peotta, Elisardo C. Vasquez and Silvana S. Meyrelles

_Hypertension_. 2001;38:555-559
doi: 10.1161/01.HYP.38.3.555

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/38/3/555

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/