Chronic Blockade of Nitric Oxide Does Not Produce Hypertension in Baroreceptor Denervated Rabbits

Rohit Ramchandra, Carolyn J. Barrett, Simon C. Malpas

Abstract—Although the vascular actions of endothelium-derived nitric oxide in modulating arterial pressure are well established, nitric oxide can also act as a neurotransmitter in the central nervous system. In addition, there is evidence for an interaction between nitric oxide and baroreceptor afferent processing; thus, nitric oxide may regulate blood pressure through central modulation of arterial baroreflexes. To test this possible interaction of nitric oxide and baroreflexes in the long-term regulation of blood pressure, we measured arterial pressure and heart rate responses to nitric oxide blockade by using L-NAME (50 mg/kg per day in drinking water) over 7 days in baroreceptor intact and sinoaortic denervated conscious rabbits. In the baroreceptor intact animals, blockade of nitric oxide leads to a significant increase in mean arterial pressure (from 75±2 to 84±3 mm Hg) and decrease in heart rate (from 233±8 to 195±8 bpm) that was sustained over the 7 days of nitric oxide blockade. In the sinoaortic denervated animals, blockade of nitric oxide initially led to a similar increase in arterial pressure (82±3 mm Hg on the second day), but in all sinoaortic denervated animals this increase was not sustained and recovered back to pre–L-NAME levels. This finding indicates that baroreflexes play an important role in the long-term control of blood pressure, and, second, that one mediator of this control is nitric oxide. (Hypertension. 2003;42:635–640.)

Key Words: baroreflex • sympathetic nervous system • blood pressure • hypertension, chronic • nitric oxide

Although the vascular actions of endothelium-derived nitric oxide in modulating arterial pressure are well established, nitric oxide can also act as a neurotransmitter in the central nervous system. Acute studies indicate a central action of nitric oxide on sympathetic nerve activity, but the role of nitric oxide in regulating long-term control of arterial pressure through a chronic action on sympathetic nerve activity has not been widely considered. The action of nitric oxide on arterial pressure through arterial baroreflexes has been considered irrelevant because baroreflexes were considered to reset with sustained increases in blood pressure. However, recent research indicates that baroreflexes may indeed play a role in the long-term regulation of blood pressure. Thrasher developed a new surgical method to produce chronic unloading of arterial baroreceptors in dogs in which the aortic baroreceptor nerves were cut and the carotid sinus was isolated from the systemic arterial pressure. Baroreceptor unloading was induced by ligation of the common carotid artery proximal to the innervated sinus. Arterial pressure was consequently increased an average of 22 mm Hg above control. Given that nitric oxide has been shown to modulate baroreceptor afferent processing at the level of the nucleus tractus solitarius and recent work by Thrasher, it follows that an important chronic action of nitric oxide on arterial pressure may be through modulation of arterial baroreflexes. To test this possible interaction of nitric oxide and the baroreflex in the long-term regulation of arterial pressure, we measured arterial pressure and heart rate responses to L-NAME administration over 7 days in baroreceptor intact and sinoaortic denervated (SAD) conscious rabbits.

Methods

Animal Preparation
Experiments were conducted on New Zealand White rabbits of either gender (mean weight 3.15±1 kg) and were approved by the University of Auckland Animal Ethics Committee. Throughout the study, the rabbits were fed daily (100 g standard rabbit pellets supplemented with hay, carrots, and apples) at 9 AM, and water was available ad libitum. The room was kept at a constant temperature (18°C) and dark-light cycle (lights on from 6 AM to 6 PM).

Surgical Procedures
All surgical procedures were performed under halothane anesthesia. Anesthesia was induced by intravenous administration of propofol (Diprivan, AstraZeneca Ltd, 10 mg/kg) followed by intubation. SAD or sham surgery was performed at least 1 week before commencing experiments. For the SAD studies, denervation of the carotid sinus nerve and the aortic depressor nerve was achieved through a ventral midline neck incision. The aortic depressor nerve was located in the cervical region between the vagus and the sympathetic trunk by using a dissecting microscope, separated free, and sectioned near its junction with the superior laryngeal nerve. Carotid baroreceptors were denervated by stripping the adventitia in the area of the carotid bifurcation, including the internal and external carotid arteries. SAD

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was adjusted so that the daily L-NAME intake was 50 mg/kg per day. L-NAME was added to the drinking water every day and by oral administration of L-NAME (Sigma) at a dose of 50 mg/kg of baseline recordings before blockade of nitric oxide was induced in animals had recovered from surgery, they were monitored for 5 days as 2-second averages for the entire experimental period. After LabVIEW graphical programming language (National Instruments) continually logged to the computer by using software written in LabVIEW graphical programming language (National Instruments) as 2-second averages for the entire experimental period. After animals had recovered from surgery, they were monitored for 5 days of baseline recordings before blockade of nitric oxide was induced by oral administration of L-NAME (Sigma) at a dose of 50 mg/kg per day. L-NAME was added to the drinking water every day and was adjusted so that the daily L-NAME intake was ~50 mg/kg per day (~± 4 mg/kg per day). After administration of L-NAME for 7 days, arterial pressure and heart rate were monitored for a further 5 days.

Statistical Analysis
The average mean heart rate, arterial pressure, and locomotor activity values for each day across the entire experimental period were analyzed by means of ANOVA (with the experimental periods being 5 days of control, 7 days of L-NAME, and 5 days of recovery). The sum of squares was completely partitioned to account for all the variability in the data. If significance was observed (P < 0.05), further appropriate post hoc tests were carried out and multiple comparisons were corrected for by Bonferroni adjustment. Data are shown as mean±SEM.

Results
Sinoaortic denervated animals had significantly greater short-term arterial pressure variability compared with control animals (coefficient of variation 0.11±0.02 in intact versus 0.15±0.01 in SAD), although overall mean pressure was not significantly different between the groups under control conditions (75±2 in intact versus 75±3 mm Hg in SAD). In the baroreceptor intact animals, blockade of nitric oxide led to a significant increase in mean arterial pressure (from 75±2 to 84±3 mm Hg) (Figure 1) and decrease in heart rate (from 233±8 to 195±8 bpm) that was sustained over the 7 days of nitric oxide blockade. In the SAD animals, blockade of nitric oxide initially led to a similar increase in arterial pressure (86±6 mm Hg on the first day) that was not significantly different from the baroreceptor intact animals. In every SAD animal, this increase in arterial pressure was not sustained and recovered back to pre-L-NAME levels. The arterial pressure was not significantly different from control values by day 5 of L-NAME treatment in the SAD animals (Figure 2). The arterial pressure variability decreased in the sham animals (coefficient of variation from 0.11±0.02 to 0.09±0.02) but increased in the SAD animals (coefficient of variation from 0.15±0.01 to 0.17±0.02) with L-NAME administration. Although the increase in arterial pressure was not sustained in SAD animals, the decrease in heart rate was maintained over the whole 7-day period of L-NAME administration (from 240±9 to 200±9 bpm). Heart rate variability was significantly greater in the sham animals during control conditions (coefficient of variation 0.11±0.02 sham versus 0.09±0.01 SAD) but increased in both groups of animals with L-NAME administration.

There was no difference in water intake, food intake, and locomotor activity between the baroreceptor intact and the SAD animals under all conditions. A circadian variation in...
arterial pressure, heart rate, and locomotor activity was evident in all animals, with an increase in arterial pressure and heart rate associated with feeding at 9 AM each day. No significant differences in the circadian variation were noted between intact and the SAD animals or during each of the experimental phases. To ensure that the changes in arterial pressure in the SAD animals were not related to the dose of L-NAME, we repeated the studies in two SAD animals with double the dose of L-NAME (100 mg/kg per day) and observed a similar lack of sustained increase in arterial pressure.

Discussion

The major finding of this study is that chronic nitric oxide blockade in SAD rabbits did not result in a chronic sustained elevation in arterial pressure. This surprising finding has several important implications for the long-term control of blood pressure. First, it suggests that baroreflexes play an important role in the long-term control of blood pressure, and, second, that one mediator of this control is nitric oxide.

Historically, it has been considered that the baroreflexes do not play a role in the chronic long-term control of blood pressure. This has been based on three main lines of evidence. First, arterial baroreceptors reset during sustained increases in arterial pressure. Second, chronic sinoaortic denervation, while producing tremendous lability in arterial pressure, does not result in higher average 24-hour pressures. Finally, it is thought that the reflex gain of the baroreceptor control system is not sufficiently strong to explain the long-term constancy of arterial pressure. Emerging evidence has, however, suggested a revision of the role of arterial baroreflexes in long-term control of blood pressure.

Lohmeier and colleagues studied responses to 5 days of angiotensin II infusion in dogs using a split-bladder preparation combined with denervation of one kidney. During angiotensin II infusion, sodium excretion from the innervated kidney significantly increased compared with the denervated kidney, indicating a decrease in renal sympathetic nerve activity (SNA). The sustained increase in sodium excretion from the innervated kidney was proposed to be mediated by baroreflex-mediated suppression of renal SNA because after cardiopulmonary and sinoaortic denervation, the sodium excretion from the innervated kidney actually decreased compared with the excretion from the denervated kidney during angiotensin II infusion. If indeed the baroreflex does play an important role in regulating long-term levels of arterial pressure, then any system that can modulate baroreflex control over arterial pressure will be important in modulating arterial pressure. We propose that nitric oxide plays an important role in chronic regulation of blood pressure by modulating baroreflex control over blood pressure.

Previous studies describe the primary action of nitric oxide on blood pressure is through an effect on the vasculature. Chronically, an action of nitric oxide in setting SNA levels and therefore blood pressure has also been indicated. The fall in blood pressure that occurs with ganglionic blockade is greater during L-NAME-induced hypertension compared with controls, suggesting that chronic L-NAME had led to an increase in SNA. Sympathectomy at 2 to 4 weeks of age in rats subsequently results in a reduced effect of L-NAME on blood pressure, suggesting that a rise in SNA was required for the full effect of L-NAME to be observed on blood pressure. However, the lack of direct long-term measurement of SNA providing supporting data has meant that such mechanisms have not been thought to be critical in regulating blood pressure chronically.

We observed that both SAD and intact animals initially displayed an increase in arterial pressure on commencing L-NAME. However, in the SAD animals, arterial pressure began to return toward control levels by day 3 of L-NAME. This initial increase in both groups is possibly due to direct vascular actions associated with the blockade of nitric oxide; however, the lack of maintenance of arterial pressure in the SAD animals suggests that a different mechanism is responsible for the chronic actions of L-NAME. This observation of a recovery in arterial pressure in the SAD animals with nitric oxide inhibition is very different from arterial pressure changes during other interventions in which chronic changes in mean arterial pressure are found to be similar in SAD and intact animals.

SAD animals did not have a sustained increase in arterial pressure with nitric oxide blockade, suggesting that the baroreflex is exerting a chronic effect over arterial pressure. This action may occur through a chronic modulation of the mean level of SNA. Although SNA was not measured in the present study, we believe it is possible to interpret and suggest mechanisms that account for the results observed. We propose that blockade of nitric oxide in intact animals results in a sympathetically mediated increase in arterial pressure, whereas in SAD animals, since the baroreflexes are no longer exerting control over arterial pressure, nitric oxide cannot influence arterial pressure through baroreflex modulation of SNA.

We undertook a number of steps to ensure that our results were specifically caused by the lack of arterial baroreflexes. Before commencing the study, denervation was verified through the absence of a decrease in heart rate with phenylephrine infusion. The increased short-term variability also confirms that the animals were denervated. Importantly, there was no difference in water intake, food intake, and locomotor activity between the baroreceptor intact and the SAD animals. The dose of L-NAME was matched to the water drunk by each animal the previous day to ensure that each animal was receiving the same dose (50 mg/kg per day) of L-NAME. Increasing the dose of L-NAME (100 mg/kg per day) did not alter the response in SAD animals.

In addition, the bradycardia observed in both groups of animals over the entire 7 days of L-NAME administration indicates that all animals had an effective blockade of nitric oxide. This bradycardia is reportedly due to a direct action of nitric oxide on heart rate. It is unlikely that our results could be explained by a change in sensitivity to nitric oxide in SAD animals because the acute increase in arterial pressure with L-NAME was similar to that recorded in the intact animals. Finally SAD also includes chemoreflex denervation, and we cannot exclude that the results seen may be reflective of chemoreflex denervation as opposed to removal of the baroreflex, as we suggest.
In conclusion, the lack of hypertension in SAD rabbits suggests an important and previously unconsidered role of nitric oxide and baroreflexes in the long-term regulation of blood pressure. Further studies that incorporate the chronic measurement of SNA will need to be conducted to elucidate the mechanisms for these interactions.

Perspectives
Our finding that SAD animals do not develop sustained hypertension with chronic nitric oxide blockade has a number of important implications for blood pressure control. This finding suggests that in addition to the arterial vasculature, the baroreflex is another important site where nitric oxide can regulate arterial pressure. Although vascular actions of nitric oxide have been studied in the past, this study is the first to demonstrate that intact baroreflexes are necessary for the chronic hypertensive actions of nitric oxide blockade. Given impaired baroreflex function is a feature of some forms of hypertension, it is possible that nitric oxide contributes to the altered blood pressure control seen in these patients. The mechanisms of this interaction between the baroreflex and nitric oxide will need to be explored further in future studies.

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