Hypertensive response to chronic NO synthase inhibition is different in Sprague-Dawley rats from two suppliers

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Pollock, David M., and Anthony Rekito. Hypertensive response to chronic NO synthase inhibition is different in Sprague-Dawley rats from two suppliers. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1719–R1723, 1998.—Experiments were conducted to determine whether Sprague-Dawley rats from different suppliers have the same hypertensive response to chronic inhibition of nitric oxide synthase. Rats (240–260 g) obtained from either Harlan or Charles River Laboratories were maintained in metabolic cages for baseline (week 0) measurements before receiving N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME) in the drinking water for 2 wk at 5 or 65 mg·kg\(^{-1}\)·day\(^{-1}\). Baseline values for tail cuff pressure (TCP) were significantly higher in Harlan rats (131 ± 2 mmHg) compared with Charles River rats (108 ± 3 mmHg, P < 0.001). At 65 mg·kg\(^{-1}\)·day\(^{-1}\), L-NAME produced a significantly larger increase in TCP in Harlan versus Charles River rats (41 ± 4 vs. 29 ± 4%, respectively, P < 0.01). Food and water intake and sodium and water excretion were not different between groups. Urinary excretion of nitrate and nitrite (U\(_{NOx}\)) was significantly reduced in all rats given L-NAME. U\(_{NOx}\) was decreased by 69 ± 12 and 62 ± 7% in Harlan and Charles River rats, respectively. The lower dose of L-NAME increased TCP and decreased U\(_{NOx}\) in both Harlan and Charles River rats; these effects were more pronounced in the Harlan rats. These results suggest that NO plays a more significant role in the maintenance of arterial pressure in Sprague-Dawley rats from Harlan compared with Charles River Laboratories. Such findings may also provide insight as to why some of the mechanisms associated with chronic L-NAME treatment are not consistent between laboratories.

N\(^{-}\)-nitro-L-arginine methyl ester; urinary nitrate and nitrite

NITRIC OXIDE (NO) is an endothelium-derived relaxing factor produced by the enzyme NO synthase (NOS). Both acute and chronic administration of inhibitors of this enzyme, such as N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME), increase vascular resistance and blood pressure when administered in vivo. Many laboratories, including our own, have conducted investigations into the various mechanisms that are involved in the response to subchronic and chronic treatment with L-NAME and how different vasoactive systems contribute to the associated hypertension (1, 6, 9). Our initial studies were conducted on Sprague-Dawley rats obtained from Charles River Laboratories (6). More recently, we began investigating the effect of L-NAME in Sprague-Dawley rats of a different supplier, Harlan Laboratories. In an attempt to reproduce our previous findings, it appeared to us that the Harlan rat was more sensitive to the hypertensive effects of L-NAME. To determine whether this possibility was true, we designed the current study to compare the hypertensive response of supposedly identical rats obtained from these two suppliers. If such a difference did exist, this might provide an explanation for why some of the responses associated with chronic L-NAME treatment are not consistent between laboratories.

METHODS

These studies used male Sprague-Dawley rats from Harlan Laboratories (Hsd:Sprague Dawley SD) and Charles River Laboratories (Crl:CD). On arrival at the Medical College of Georgia, Department of Laboratory Animal Services, all rats had an initial body weight ranging from 200 to 225 g. Rats of this weight differed in age from these two suppliers; Harlan rats were 6.5 wk, whereas Charles River rats were 7.9 ± 0.6 days old (–8 wk) despite being similar in size. Rats were allowed to acclimate to individual housing for 1 wk before the beginning of the experiments. Baseline measurements of food and water intake and excretory function were obtained by placing the rats in metabolic cages for 2 days. This was followed by measurement of tail cuff pressures as an estimate of systolic arterial pressure, as described previously (6, 7). In brief, rats were warmed in a restraining chamber and occluding cuffs and pneumatic pulse transducers were placed on the rats’ tails. A programmed electrophysiomonometer automatically inflated and deflated the tail cuff while signals from the transducer were automatically collected using a MacLab (ADInstruments, Milford, CT) connected to a Macintosh Power PC. Twelve readings were taken for each rat, the highest and lowest and any associated with excess noise or animal movement were discarded, and then the readings were averaged to determine systolic arterial pressure.

After baseline measurements, rats were then given tap water containing L-NAME (Sigma, St. Louis, MO) to drink ad libitum at a concentration to deliver either 5 or 65 mg·kg\(^{-1}\)·day\(^{-1}\). The concentration of L-NAME was adjusted on a daily basis to ensure proper dosing. Control rats were given tap water. Measurements of tail cuff pressure, intake, and excretory variables were again obtained during each of
the next 2 wk, during which L-NAME treatment continued. At the end of the 2-wk period, rats were anesthetized with pentobarbital sodium (65 mg/kg ip) and a terminal blood sample was taken from the abdominal aorta to measure creatinine and electrolyte concentrations. The numbers of rats in each group were as follows: 12 control Charles River rats, 6 Charles River rats given L-NAME at 5 mg·kg\(^{-1}\)·day\(^{-1}\), 9 Charles River rats given L-NAME at 65 mg·kg\(^{-1}\)·day\(^{-1}\), and 6 in each of the 3 groups of Harlan rats.

A Beckman EL-ISE analyzer was used to measure sodium and potassium concentrations in all of the urine and plasma samples. Urine and plasma creatinine concentrations were measured by the picric acid colorimetric method and were then used to determine creatinine clearance as an estimate of glomerular filtration rate (GFR). Total urinary nitrate and nitrite (NO\(_x\)) was determined using the Greiss reaction (7). Values reported are means ± SE. ANOVA for repeated measures with a univariate model and post hoc contrasts was used to determine significant differences between individual means (SuperANOVA; Abacus Concepts, Berkeley, CA). Regression analysis was conducted to evaluate the relationship between basal systolic pressure and the change in pressure produced by L-NAME (GraphPad Software, San Diego, CA).

RESULTS

Baseline tail cuff pressures (week 0) of Sprague-Dawley rats obtained from Harlan and Charles River Laboratories were significantly different despite the similar size of the rats; Harlan rats averaged 131 ± 2 mmHg (n = 18), whereas Charles River rats averaged 108 ± 3 mmHg (n = 27). Treatment of rats with L-NAME at 65 mg·kg\(^{-1}\)·day\(^{-1}\) increased tail cuff pressure in the Harlan rats by 41 ± 4% above baseline over the 2-wk treatment period (Fig. 1). In the Charles River rats, the increase in tail cuff pressure was only 29 ± 4% when they underwent the identical treatment. In both Harlan and Charles River rats, there was a significant negative correlation between the basal pressure and the increase in pressure that occurred over the 2-wk treatment period. The slopes of the regression lines were nearly identical: −1.08 ± 0.34 for Harlan rats (r\(^2\) = 0.72, P = 0.03) and −1.06 ± 0.33 for Charles River rats (r\(^2\) = 0.59, P = 0.01).

In separate groups of rats, L-NAME given at 5 mg·kg\(^{-1}\)·day\(^{-1}\) produced smaller changes in tail cuff pressure (Fig. 2). A significant increase in tail cuff pressure was noted after 1 wk of L-NAME in Harlan but not in Charles River rats. After 2 wk of treatment, L-NAME increased tail cuff pressure by 17 ± 2% in Harlan rats and 13 ± 4% in rats from Charles River; this difference was not statistically significant.

The urinary excretion of nitrate and nitrite (U\(_{NOx}\), used to evaluate the degree of NOS inhibition, was decreased in rats from both sources when they were given L-NAME (Fig. 3). L-NAME at the dose of 65 mg·kg\(^{-1}\)·min\(^{-1}\) decreased U\(_{NOx}\) by 71 ± 12% in rats from Harlan but only by 62 ± 7% in Charles River rats. At 5 mg·kg\(^{-1}\)·day\(^{-1}\) L-NAME, Harlan rats displayed a greater sensitivity to L-NAME (Fig. 4). After 1 wk of treatment, U\(_{NOx}\) was significantly decreased by L-NAME in Harlan rats but not significantly changed in Charles River rats. At week 2, U\(_{NOx}\) was again significantly lower in Harlan rats. Calculated as percent change from baseline, however, the decrease at 2 wk...
was not significantly different between groups (45 ± 7% for Harlan and 31 ± 2% for Charles River, P = 0.084).

The high dose of L-NAME, 65 mg·kg⁻¹·day⁻¹, had no effect on body weight of rats from either source (Table 1). Charles River rats weighed significantly more than Harlan rats after the 2-wk study period, although this may be related to the difference in age. Food and water intake were similar between rats from both sources, and L-NAME had no effect on these variables. Urinary volume was similar between groups, with the exception that Harlan rats given L-NAME had a significantly greater urine volume after 2 wk of treatment. L-NAME also had no effect on sodium excretion in either Harlan or Charles River rats.

**DISCUSSION**

These experiments establish several potentially important differences between Sprague-Dawley rats from two major supply houses. First, the baseline arterial pressures were consistently and significantly higher in Harlan rats after the 2-wk study period, although this may be related to the difference in age. Food and water intake were similar between rats from both sources, and L-NAME had no effect on these variables. Urinary volume was similar between groups, with the exception that Harlan rats given L-NAME had a significantly greater urine volume after 2 wk of treatment. L-NAME also had no effect on sodium excretion in either Harlan or Charles River rats.

**Fig. 3.** Effect of L-NAME at 65 mg·kg⁻¹·day⁻¹ on urinary nitrate and nitrite excretion (UNOX) in Harlan (A) and Charles River (B) Sprague-Dawley rats. *P < 0.05 vs. control rats from same supplier.

**Table 1.** Effect of L-NAME at 5 or 65 mg·kg⁻¹·day⁻¹ on body weight, food and water intake, and urinary excretion in Harlan and Charles River Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Body weight, g</th>
<th>Food intake, g/day</th>
<th>Water intake, ml/day</th>
<th>Urine volume, ml/day</th>
<th>Sodium excretion, mmol/day</th>
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<tr>
<td>0</td>
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<td>2</td>
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<tr>
<td>Body weight, g</td>
<td>243 ± 4</td>
<td>286 ± 6</td>
<td>315 ± 7</td>
<td>245 ± 6</td>
<td>286 ± 6</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>23.5 ± 0.5</td>
<td>24.1 ± 0.6</td>
<td>23.2 ± 0.8</td>
<td>22.7 ± 0.9</td>
<td>23.7 ± 0.8</td>
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<tr>
<td>Water intake, ml/day</td>
<td>28.8 ± 1.3</td>
<td>35.5 ± 2.5</td>
<td>32.9 ± 2.3</td>
<td>29.8 ± 0.9</td>
<td>33.4 ± 3.2</td>
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<tr>
<td>Urine volume, ml/day</td>
<td>13.3 ± 0.6</td>
<td>12.4 ± 1.0</td>
<td>16.2 ± 1.0</td>
<td>13.7 ± 0.5</td>
<td>13.8 ± 0.6</td>
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<td>Sodium excretion, mmol/day</td>
<td>1.25 ± 0.13</td>
<td>1.10 ± 0.13</td>
<td>1.39 ± 0.17</td>
<td>0.87 ± 0.06</td>
<td>0.87 ± 0.10</td>
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Values are means ± SE. L-NAME, N⁷- nitro-arginine methyl ester. Numbers in parentheses are mg·kg⁻¹·day⁻¹ L-NAME. *P < 0.05 vs. control rats from same supplier; †P < 0.05 vs. similarly treated Harlan rats.

**Fig. 4.** Effect of L-NAME at 5 mg·kg⁻¹·day⁻¹ on urinary nitrate and nitrite excretion in Harlan and Charles River Sprague-Dawley rats. *P < 0.05 vs. week 0; †P < 0.05 vs. similarly treated Charles River rats.
Harlan rats compared with Charles River rats. We also observed that the hypertensive response to chronic L-NAME treatment was greater in the Harlan compared with the Charles River rats. Admittedly, it is unclear presently how these differences may influence some of the observations made by the various investigators using the chronic L-NAME model. Nonetheless, we can speculate that the mechanisms responsible for increasing arterial pressure during chronic NOS inhibition are different between these two groups of Sprague-Dawley rats.

It does not appear as though the differences in basal arterial pressure could account for the different hypertensive responses to L-NAME. Charles River rats had lower basal arterial pressure and a smaller change in pressure. However, within a given type of rat, whether it be Harlan or Charles River, we observed that the pressor response to L-NAME was inversely correlated with the basal pressure value. A similar correlation was reported using acute L-NAME administration (10), and so one would have predicted that Charles River rats would have had the larger response to L-NAME in our studies. Therefore the difference in basal arterial pressure between Harlan and Charles River rats may have actually minimized the differences we observed in the response to L-NAME. In addition to the higher baseline arterial pressure, Harlan rats also tended to have a lower basal urinary NOx excretion compared with Charles River rats. The latter difference was significant in the series of experiments examining the lower dose of L-NAME. Collectively, these results suggest that Harlan rats may have a defective or altered NO system that could account for the higher basal arterial pressure.

Originally, Sprague-Dawley rats from both suppliers came from a single colony of rats. Over the years, there has been no genetic crossbreeding among suppliers or even among different breeding locations for a single supplier. There are also some differences in breeding procedures between the two suppliers that could account for some of the genetic divergence. Harlan uses a totally random method for selecting breeders with no regard to the size of litter from which they were born. Because they choose offspring from a very large pool of breeders, there is only a small chance of brother-sister mating. Charles River, on the other hand, has traditionally selected their breeders based on litter size in an effort to maximize the number of pups obtained from their breeding pairs. They never choose breeders from litters of fewer than 8 pups. Neither supplier crossbred rats from their different breeding facilities, so it is also possible that within a given supplier, genetic variations could exist depending on where they were bred. It is interesting that since the time that the current study was completed, Charles River announced a major change in their breeding procedures to minimize inbreeding, genetic drift, and colony divergence by systematic outbreeding and animal migration among their many breeding locations. Among these changes are that only one pup per litter will be chosen as a future breeder and that there will be no selection based on minimum litter size.

Given the differences in breeding procedures in the absence of any crossbreeding, it is quite probable that genetic differences have developed in these colonies that now result in alterations in the balance of factors that determine arterial pressure and the response to L-NAME. More specifically, the degree to which NO contributes to maintaining basal arterial pressure can be significantly different among groups of otherwise “normal” rats. We also noted that the rats were different in age for the weight range that we studied. The Harlan rats were >1 wk younger than the Charles River rats despite being similar in size. It is unclear whether such a difference could account for the observed changes in response to L-NAME. However, Hill et al. (3) examined the acute hypertensive response to L-NAME in rats at 3–5 versus 18–21 mo of age. These investigators reported that the pressor response to NOS inhibition was similar in young versus old rats despite the latter group having a higher basal arterial pressure. To date, there have been no studies examining age-related differences in response to chronic L-NAME treatment.

Among the investigations that have studied the pressor and hemodynamic changes that occur during chronic NOS inhibition, there have been a number of findings that have not been consistent between laboratories. For example, Hu et al. (4) reported that chronic L-NAME hypertension is not reversible. That is, the hypertension persists even after L-NAME administration is discontinued. In contrast, Pollock et al. (6) observed that L-NAME hypertension is rapidly reversible. The former group used Harlan Sprague-Dawley rats, whereas the latter used rats from Charles River. Another set of contrasting findings has been related to the degree and/or time course of renal failure that occurs during chronic L-NAME. Although most investigators have reported reduced GFR beginning almost immediately after initiation of L-NAME treatment (4, 8, 9), others have noted very little change even after 4 wk of treatment (2, 5, 6). Such differences could easily be ascribed to the differences in the extent of vasoconstriction associated with NOS inhibition. Interestingly, the severity of renal damage associated with L-NAME hypertension has been linked to genetic variables and not to the degree of hypertension (11).

There are a number of other mechanisms that have been investigated and proposed to contribute to the hypertension associated with L-NAME. These include ANG II, sympathetic nerve activity, dietary sodium intake, and so forth. Subtle differences in one or more of these systems could account for the differences we observed in Sprague-Dawley rats from these two sources. Consequently, our current findings, investigators exploring the various mechanisms related to L-NAME hypertension must now consider the possibility that the source of rats is a potentially conflicting factor when making comparisons to previously published work.
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