Nitric oxide modulates arterial baroreflex control of heart rate in conscious lambs in an age-dependent manner

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Sener, Alp, and Francine G. Smith. Nitric oxide modulates arterial baroreflex control of heart rate in conscious lambs in an age-dependent manner. Am J Physiol Heart Circ Physiol 280: H2255–H2263, 2001.—Experiments were carried out in conscious chronically instrumented lambs aged 1 (n = 6) and 6 wk (n = 5) to evaluate the arterial baroreflex control of heart rate (HR) during postnatal maturation and to investigate any modulatory role of endogenously produced nitric oxide (NO). Before and after intravenous administration of 20 mg/kg of the L-arginine analog N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME), the arterial baroreflex was assessed by measuring HR responses to increases and decreases in systolic arterial pressure achieved by intravenous administration of phenylephrine and sodium nitroprusside. The HR range over which the baroreflex operates and minimum HR as well as maximum gain were greater at 1 than at 6 wk of age. These age differences were abolished in the presence of L-NAME, which decreased the HR range and gain of the arterial baroreflex control of HR at 1 but not at 6 wk of age. These data provide new information that age-dependent effects of the arterial baroreflex appear to result from effects of endogenously produced NO.

newborn; perinatal; \(\text{N}^{\text{G}}\)-nitro-L-arginine methyl ester; blood pressure

The arterial baroreceptor reflex plays an important role in maintaining cardiovascular homeostasis by continuously monitoring second-to-second changes in blood pressure. Afferent information from arterial baroreceptors (located in the carotid sinus and aortic arch regions) is relayed to the nucleus tractus solitarius (NTS) of the hindbrain, where it is processed and integrated. Arterial baroreflex-mediated alterations in heart rate (HR) and sympathetic nervous activity act to compensate for any perturbations in the system (9, 46).

There is considerable interspecies variability in the age of onset of the arterial baroreceptor reflex. In pigs (5), rabbits (52), and dogs (51), baroreceptor sensitivity is reported to be low at birth, increasing with postnatal age. In the developing rat, a mature baroreceptor reflex is not present until the third week of postnatal life, at which time sympathetic pathways have developed (2).

As well, activity of baroreceptor afferents has been demonstrated during fetal life in sheep (3) and in newborn rabbits (11, 48). It is generally agreed that the arterial baroreflex regulation of HR is developmentally regulated, although there is discrepancy with respect to developmental changes in the parameters governing the arterial baroreflex. As outlined in Table 1, the sensitivity of the arterial baroreflex has been shown to decrease (48), increase (10, 16, 23, 29, 51), or remain unchanged (40) during maturation. These discrepancies may result from differences in species as well as in experimental design, including the state of the animal (e.g., anesthetized, sedated and paralyzed, or conscious), the method of altering HR (e.g., aortic balloon inflation or pharmacological manipulation of pressure), choice of drug administered (e.g., methoxamine or nitroglycerin), and method of assessing the response (e.g., maximum response, percent change, averaging >10 mmHg, or linear regression). Surprisingly, there has been no study in which the various parameters of the arterial baroreflex have been assessed during postnatal maturation using the entire range of the arterial baroreflex (i.e., after both increases and decreases in arterial pressure) in conscious undisturbed animals.

In recent experiments (41, 42) carried out in conscious chronically instrumented lambs trained to the laboratory environment, we investigated some of the cardiovascular effects of endogenously produced nitric oxide (NO) by measuring hemodynamic effects of the L-arginine analog \(\text{N}^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME) during postnatal maturation. In the presence of L-NAME, mean arterial pressure increased to a similar extent in 1- and 6-wk-old animals, yet the concomitant decrease in HR was considerably greater in lambs aged 1 wk compared with the older lambs. These previous findings provided evidence to suggest that NO may normally modulate the arterial baroreflex in newborn lambs and formed the rationale for the present study.

The current experiments were therefore an extension of our previous aforementioned findings and were designed to test the hypothesis that NO modulates the arterial baroreflex control of HR in an age-dependent manner.
manner. To test this hypothesis, we first assessed the arterial baroreflex control of HR at two postnatal ages in conscious chronically instrumented lambs to determine whether the parameters governing this relationship are altered with postnatal maturation. Second, we investigated the role of endogenously produced NO in modulating the various parameters of the arterial baroreflex control of HR at two postnatal ages in conscious chronically instrumented lambs aged ~1 wk (6–11 days, n = 6, 6.6 ± 0.4 kg body wt) and ~6 wk (41–44 days, n = 5, 12.5 ± 1.8 kg body wt). Animals were obtained from a local source (Sheep Advisory Service; Alberta, Canada) and housed with their mothers in individual pens in the vivarium of the University of Calgary Health Sciences Centre except during surgery and experiments. All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

Surgical procedures. Under halothane anesthesia and with the use of sterile techniques, surgery was performed for placement of catheters in femoral vessels using techniques previously described by us (41, 44). Polyvinyl catheters (polyethylene-160) were advanced to the abdominal aorta and inferior vena cava for recording of arterial pressures and intravenous infusions and injections of drugs during experiments. Catheters were tunneled subcutaneously to exit the lamb on the right and left flanks, where they were stored in pouches on a lamb body jacket (Lomir; Montreal, Canada). Lambs were allowed to recover from the effects of surgery and anesthesia in a critical care unit for small animals (Shor-line, Schroer Manufacturing; Kansas City, MO) with an adjustable oxygen supply. All lambs were able to stand soon after the completion of surgery, at which time they were returned to the vivarium, where they were housed with their mothers until the time of the experiment. Antibiotics (0.5 mg/kg enrofloxacin, Baytril) were administered intramuscularly at surgery and at 12-h intervals thereafter for 48 h. During the recovery period, lambs were trained to rest quietly in a supportive sling in the laboratory environment to allow them to become accustomed to their surroundings.

Experimental details. On the day of an experiment, the lamb was removed from the vivarium and placed in the same supportive sling in the laboratory environment to allow them to become accustomed to their surroundings.

Table 1. Summary of baroreflex studies during development

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference Number</th>
<th>Age</th>
<th>State</th>
<th>Baroreflex assessment</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>11</td>
<td>Newborns 0–15 days, ≥3 wk</td>
<td>Anesthetized</td>
<td>Aortic depressor and carotid sinus nerve stimulation</td>
<td>With increasing age, threshold for activation of baroreceptors increases</td>
</tr>
<tr>
<td>Sheep</td>
<td>43</td>
<td>Fetuses</td>
<td>Conscious</td>
<td>Aortic balloon inflation</td>
<td>Increasing baroreflex sensitivity with advancing gestational age</td>
</tr>
<tr>
<td>Dog</td>
<td>51</td>
<td>Newborns 1–7 days, 5–7 wk, adults</td>
<td>Conscious</td>
<td>HR response to intravenous methoxamine</td>
<td>Depressed baroreflex sensitivity in newborns compared with adults</td>
</tr>
<tr>
<td>Sheep</td>
<td>29</td>
<td>Newborns 3–15 days, adults</td>
<td>Conscious</td>
<td>HR response to intravenous methoxamine</td>
<td>Depressed baroreflex sensitivity in newborns compared with adults</td>
</tr>
<tr>
<td>Sheep</td>
<td>10</td>
<td>Fetuses &lt;142 days, newborns 2–23 days, adults</td>
<td>Conscious</td>
<td>Aortic balloon inflation and HR response to intravenous methoxamine or phenylephrine</td>
<td>Baroreflex depressed in fetuses and newborns compared with adults</td>
</tr>
<tr>
<td>Rabbit</td>
<td>48</td>
<td>Newborn 9–11 days, adults</td>
<td>Anesthetized</td>
<td>Carotid sinus nerve recordings and changes in perfusion pressure</td>
<td>Lower threshold pressures and higher gains in newborns than adults</td>
</tr>
<tr>
<td>Dog</td>
<td>16</td>
<td>Newborns 6–10 wk, adults</td>
<td>Anesthetized</td>
<td>HR response at 20–30 s after intravenous phenylephrine and nitroglycerin</td>
<td>Depressed baroreflex sensitivity in newborns compared with adults</td>
</tr>
<tr>
<td>Sheep</td>
<td>3</td>
<td>Fetuses newborns 7 days, 4–6 wk</td>
<td>Anesthetized</td>
<td>Carotid sinus nerve recordings and changes in perfusion pressure</td>
<td>Resetting of baroreceptors and decrease in baroreflex sensitivity with age</td>
</tr>
<tr>
<td>Sheep</td>
<td>23</td>
<td>Fetuses, adults</td>
<td>Conscious</td>
<td>HR response to intravenous angiotensin II or phenylephrine</td>
<td>Depressed baroreflex sensitivity in fetuses compared with adults</td>
</tr>
<tr>
<td>Sheep</td>
<td>40</td>
<td>Fetuses &lt;140 days, newborns 3–7 days, 4–6 wk</td>
<td>Sedated and paralyzed</td>
<td>Maximum HR response to intravenous phenylephrine and nitroglycerin as percentage of control</td>
<td>Decreased baroreflex sensitivity and higher threshold pressures in lambs compared with fetuses</td>
</tr>
</tbody>
</table>

HR, heart rate.
simultaneously digitized at 200 Hz to a computer using the data acquisition and analysis software package CVSOFT (Odessa Systems). The right femoral venous catheter was used to administer drugs during experiments.

Two experiments were carried out in each animal at intervals of 24 to 48 h and in random order. Experiment 1 consisted of assessment of the arterial baroreflex control of HR before (control) and at 30-min intervals for 2 h after intravenous administration of 20 mg/kg of L-NAME. The dose of L-NAME was selected from our previous dose-response studies (41, 42) as that which increases systolic arterial pressure (SAP) for 120 min; the effects being similar in both age groups. Experiment 2 consisted of assessment of the arterial baroreflex control of HR before (control) and at 30-min intervals for 2 h after intravenous administration of 20 mg/kg of D-NAME. In both experiments, the arterial baroreflex control of HR was assessed as follows: phenylephrine hydrochloride (10 μg/kg, Sabex) and sodium nitroprusside (Nipride, 10 μg/kg, Hoffman-LaRoche) were infused intravenously over 5 s to increase and decrease arterial pressure, respectively, from resting levels. Doses of phenylephrine and sodium nitroprusside were chosen from previous dose-response curves carried out in our laboratory to increase and decrease systolic arterial pressure by −25–30 mmHg to allow the construction of the physiological range over which the arterial baroreflex operates, including the upper and lower limits. Beat-to-beat SAP as well as pulse intervals were measured for 10 s before each intervention, and measurements were continued until the maximum response was achieved. The relationship between SAP and HR obtained at the consecutive pulse interval was constructed for each animal. Data obtained from each age group were pooled, and a four-parameter sigmoid logistic function curve was applied (SigmaPlot, version 5.0, Jandel Scientific) to both sets of data at the following time points: control and 30, 60, 90, and 120 min. The method used to assess the arterial baroreflex control of HR was that described by Kent et al. (26) as follows: HR = P1 + P2 × [1 + exp (P3 × SAP − P4)], where P1 is the HR range, P2 is the slope coefficient, P3 is SAP at the midpoint of the HR range, and P4 is minimum HR. Maximum gain (Gmax) was also that described by Kent et al. (26) (Gmax = −P1 × P3 × 0.25) and was considered to describe the sensitivity of the baroreflex control of HR (see also Ref. 24).

For parameters governing the arterial baroreflex control of HR, statistical comparisons between 1- and 6-wk-old lambs were made using Student’s nonpaired t-tests and between time points of control and 30, 60, 90, and 120 min using Student’s paired t-tests with a Bonferroni correction. For SAP and HR, statistical comparisons were determined by applying ANOVA for repeated measures; the factors were age (1 vs. 6 wk) and drug (L-NAME vs. D-NAME). Significance was accepted at the 95% confidence interval.

RESULTS

Figure 1A illustrates raw data representing the arterial baroreflex control of HR measured during baseline; in Fig. 1B, the logistic function is applied to the raw data shown in Fig. 1A. There was a significant decrease in the HR range over which the arterial baroreflex operates in 6-wk-old compared with 1-wk-old lambs and a decrease in minimum HR. SAP at the midpoint HR range and slope coefficient were similar at 1 and 6 wk of age, although the maximum gain was greater in lambs aged 1 wk compared with 6 wk (see also Table 3).

Table 2. Cardiovascular effects of L-NAME in conscious 1- and 6-wk-old lambs

<table>
<thead>
<tr>
<th>L-NAME (20 mg/kg iv)</th>
<th>Age</th>
<th>Control</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td></td>
<td>109 ± 10</td>
<td>121 ± 13*</td>
<td>116 ± 7</td>
<td>120 ± 15*</td>
<td>119 ± 10*</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td>111 ± 9</td>
<td>118 ± 15</td>
<td>124 ± 9*</td>
<td>124 ± 9*</td>
<td>122 ± 11*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td></td>
<td>198 ± 28</td>
<td>155 ± 16*</td>
<td>144 ± 19*</td>
<td>148 ± 13*</td>
<td>153 ± 18*</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td>124 ± 9*</td>
<td>105 ± 21†</td>
<td>106 ± 23†</td>
<td>103 ± 13†*</td>
<td>113 ± 21†</td>
</tr>
</tbody>
</table>

Values are means ± SD. SAP, systolic arterial pressure; L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 compared with control; †P < 0.05 compared with 1-wk-old lambs.
of L-NAME administration; effects were more predominant at 1 wk of age (Table 2).

In 1-wk-old lambs, 30 min after administration of L-NAME, there was a significant decrease in the HR range over which the arterial baroreflex operates and a decrease in minimum HR (Fig. 2 and Table 3); these effects were sustained for 90 min (Fig. 2). L-NAME also decreased the slope coefficient at 60 min and signifi-

Table 3. Effects of L-NAME on parameters governing arterial baroreflex

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR range, beats/min</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 wk</td>
<td>177 ± 32</td>
<td>117 ± 33*</td>
<td>151 ± 39*</td>
<td>114 ± 16*</td>
<td>132 ± 18</td>
</tr>
<tr>
<td>6 wk</td>
<td>91 ± 12†</td>
<td>132 ± 28</td>
<td>112 ± 21</td>
<td>115 ± 33</td>
<td>144 ± 54</td>
</tr>
<tr>
<td></td>
<td>Slope of coefficient</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 wk</td>
<td>0.07 ± 0.3</td>
<td>0.06 ± 0.1</td>
<td>0.04 ± 0.1*</td>
<td>0.07 ± 0.4</td>
<td>0.07 ± 0.3</td>
</tr>
<tr>
<td>6 wk</td>
<td>0.06 ± 0.3</td>
<td>0.05 ± 0.2</td>
<td>0.05 ± 0.2</td>
<td>0.05 ± 0.2</td>
<td>0.05 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>SAP at midpoint range, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>123 ± 3</td>
<td>138 ± 8*</td>
<td>126 ± 6*</td>
<td>123 ± 4</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>6 wk</td>
<td>116 ± 3†</td>
<td>116 ± 6†</td>
<td>112 ± 6</td>
<td>117 ± 9</td>
<td>111 ± 9</td>
</tr>
<tr>
<td></td>
<td>Minimum HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>103 ± 18</td>
<td>58 ± 26*</td>
<td>75 ± 19*</td>
<td>97 ± 9</td>
<td>104 ± 9</td>
</tr>
<tr>
<td>6 wk</td>
<td>72 ± 7†</td>
<td>51 ± 10</td>
<td>68 ± 8</td>
<td>60 ± 10†</td>
<td>52 ± 22†</td>
</tr>
<tr>
<td></td>
<td>Maximum gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>−2.48 ± 1.6</td>
<td>−1.72 ± 1.2*</td>
<td>−1.58 ± 1.0*</td>
<td>−2.00 ± 1.8*</td>
<td>−2.49 ± 1.5</td>
</tr>
<tr>
<td>6 wk</td>
<td>−1.64 ± 0.9†</td>
<td>−1.66 ± 1.0</td>
<td>−1.59 ± 0.9</td>
<td>−1.50 ± 0.8†</td>
<td>−1.84 ± 1.0†</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 compared with control; †P < 0.05 compared with 1-wk-old lambs.
sstantly decreased the maximum gain of the SAP-HR relationship at 30–90 min. In fact, at 30–60 min after L-NAME administration, there was little difference between 1- and 6-wk-old lambs in any of the parameters governing the arterial baroreflex. There was also a transient increase in the SAP at the midpoint HR range 30 min after administration of L-NAME to 1-wk-old lambs; this effect was not sustained.

In 6-wk-old lambs, there were no apparent effects of L-NAME on any of the parameters of the arterial baroreflex measured at 30–120 min (Fig. 3 and Table 3).

There were no significant effects of D-NAME on the arterial baroreflex control of HR in either age group of lambs.

**DISCUSSION**

The purpose of the present study was to investigate the role of NO in modulating the arterial baroreflex control of HR under normal physiological conditions during postnatal maturation. Our findings provide new information that the arterial baroreflex control of HR operates over a wider HR range in the newborn period compared with later in life, and the sensitivity of this relationship decreases as maturation proceeds. These data also provide evidence that the arterial baroreflex shifts toward a lower set point for resting HR as maturation proceeds. Age-dependent differences in the arterial baroreflex control of HR appear to result from endogenously produced NO, because they are abolished in the presence of the L-arginine analog L-NAME but not its inactive isomer D-NAME. Therefore, we accept our hypothesis that NO modulates the arterial baroreflex control of HR in an age-dependent manner.

Numerous investigations have been performed to evaluate the arterial baroreflex control of HR during development; these are outlined in Table 1. The results from these studies are, however, often confounded by various factors including allowing some of the animals to sleep during the experiments (this has been shown to alter the reflex control of HR and blood pressure; see Refs. 13 and 45) or drawing conclusions about the entire range of the arterial pressure-HR relationship after assessing only one portion. To our knowledge, no study has been carried out to investigate the arterial baroreflex control of HR over the entire range of the SAP-HR relationship under normal physiological conditions during postnatal maturation. Our study is, therefore, the first to describe the maturational changes in the arterial baroreflex control of HR during postnatal development in conscious undisturbed animals fully trained to the laboratory environment. We

![Fig. 3. Arterial baroreflex control of HR in conscious lambs aged 6 wk measured during control (solid line) and at 30 min (A), 60 min (B), 90 min (C), and 120 min (D) after L-NAME administration (gray line). Four-parameter logistic function was applied to raw data.](image-url)
found that there was a decrease in the maximum gain of the arterial baroreflex as maturation progressed as well as in the HR range over which the baroreflex operates, whereas there was no significant difference in SAP at the midpoint of the HR range in 1- and 6-wk-old lambs. Therefore, as postnatal maturation proceeds, the set point for resting HR decreases. This is perhaps not surprising because resting HR also decreases over this developmental period (see also Table 2). A decreased set point with advancing age may reflect an adaptation to the normal physiological changes that occur during postnatal maturation. Our findings are in agreement with the resetting of the arterial baroreceptors themselves observed from experiments by Blanco et al. (3), in which carotid sinus nerve activity was directly recorded at various stages of fetal and newborn development in sheep. In addition, our findings suggest that, in the immediate newborn period, the arterial baroreflex is normally operating at its upper range, whereas later in life, when resting HR is lower, the arterial baroreflex appears to be operating midrange. This may have important implications for age-dependent differences in the physiological responses to changes in blood pressure and/or vascular volume.

Recently, Mayhan (31) has shown that inhibition of NO synthase (NOS) (with the use of drugs such as L-NAME) does not alter the basal permeability of the blood-brain barrier, at least in rats. Unlike L-arginine and other arginine analogs, L-NAME does not appear to be transported across the blood-brain barrier using the endothelial amino acid uptake system but rather relies on simple diffusion to enter the cytosol (4). This means that some time is needed for L-NAME to enter into the central nervous system (CNS) to exert its inhibitory effects on brain NOS activity. In fact, Iadecola et al. (22) showed that intravenous administration of 20 mg/kg of L-NAME to Sprague-Dawley rats attenuated brain NOS activity, as assessed by the conversion of L-arginine to L-citrulline, within 30 min; this effect was sustained for at least 2 h, and no additional attenuation of brain NOS activity occurred at doses >20 mg/kg. Similarly, Traystman et al. (49) demonstrated that within 30 min of intravenous injection of 20 mg/kg L-NAME, brain NOS activity was inhibited by 70% in pentobarbital sodium-anesthetized dogs and pigs and 90% in cats; these effects were sustained for 6 h. Assuming that the same holds true for the sheep, we can be reasonably certain that brain NOS activity was inhibited before the first measurement of the arterial baroreflex control of HR at 30 min and that this effect was sustained throughout the 2-h period of study.

In previous experiments (41, 42), we showed that administration of 20 mg/kg L-NAME was associated with a marked increase in mean arterial pressure in lambs aged 1 and 6 wk of postnatal life along with an age-dependent decrease in HR; the decrease in HR was greater at 1 wk and was sustained for 4 h after administration of L-NAME, whereas at 6 wk the HR response was considerably less and control levels were reached more quickly (42). These findings were also confirmed in the present study (see Table 2) and provide evidence that the arterial baroreflex was reset toward a lower resting blood pressure in lambs aged 1 wk after removal of endogenously produced NO. Because SAP increased within 30 min of L-NAME administration and remained elevated for the 2-h study in both age groups, we cannot rule out the possibility of acute resetting of the arterial baroreceptors towards the higher resting pressures (7, 19, 33). Inspection of our SAP-HR relationship measured during baseline conditions (control; Fig. 1) demonstrates that an increase in SAP of ~10 mmHg from ~110 mmHg (such as that which occurred after L-NAME administration to both age groups; see Table 2) would be associated with a decrease in HR of ~15–20 beats/min at 6 wk and ~25–30 beats/min at 1 wk. If acute resetting of the arterial baroreflex towards higher pressures had occurred after L-NAME administration, the concomitant decrease in HR would be less. This was not the case (see Table 2). Therefore, it seems unlikely that there was any acute baroreceptor resetting toward higher pressures after L-NAME administration, although additional studies are warranted to confirm this.

When we assessed the arterial baroreflex after administration of L-NAME (Table 3), various parameters governing the arterial baroreflex were altered in lambs aged 1 wk such that the arterial baroreflex appeared to assume that of 6-wk-old animals at 30 and 60 min. In 1-wk-old lambs, at 120 min, the arterial baroreflex control of HR began to return toward control levels. The reason for this return toward control by 120 min is not known but may reflect secondary effects of L-NAME on other humoral systems that regulate the arterial baroreflex early in life (e.g., a decrease in angiotensin II). Interestingly, there were no significant changes in any of the parameters governing the arterial baroreflex after L-NAME administration to conscious 6-wk-old lambs at any of the time points studied. Parameters governing the arterial baroreflex are modulated by changes in afferent, central, and efferent components of the baroreflex arc. Because the NO system exists at all of these sites (53), effects of L-NAME on the arterial baroreflex could be occurring anywhere in the arterial baroreflex arc, including the CNS. Previous investigations have attempted to address these three components of the baroreflex arc. These are detailed in the preceding paragraphs, which are focused primarily on studies in conscious animals to avoid the known impact of surgery and anesthesia on the NO system (24, 28, 47) and because the arterial baroreflex is considerably altered in the presence of anesthesia (1, 50).

In anesthetized rats, Matsuda et al. (30) injected the NO-related nitrosothial compound S-nitrosocysteine (cysNO) as well as thimerosal, an inhibitor of acyl CoA and a powerful stimulator of NO release from endothelial cells, into the isolated carotid sinus. A decrease in the activity of the carotid sinus nerve was recorded after both cysNO and thimerosal, suggesting a direct inhibitory effect of NO on neuronal excitability of arterial baroreceptors. Age-dependent effects of NO on
the arterial baroreflex control of HR observed in the present study could therefore be occurring through an age-dependent effect of NO on the arterial baroreceptors. However, treatment of the isolated carotid sinus with L-NAME or with hemoglobin has no effect on baroreceptor activity, suggesting that endogenously released NO does not normally modulate the arterial baroreceptors directly (30). Therefore, it is unlikely that our findings reflect an age-dependent effect of NO on arterial baroreceptor afferent activity.

In the CNS, NO modulates both HR and sympathetic outflow through N-methyl-D-aspartate receptors coupled to NOS activation in the NTS (54) through the paraventricular nucleus (PVN) via a γ-amino butyric acid mechanism (55) and through cardioinhibitory neurons located within the nucleus ambiguous. Age-dependent effects of NO on the arterial baroreflex could therefore result from age-dependent changes in the localization of NOS within the PVN and NTS. In support of this premise is the recent evidence obtained in rats by Gozal et al. (15) of an age-dependent increase in neuronal NOS in the lateral segmental field of the medulla, a region intimately involved in arterial baroreflex regulation of HR. Studies by Northington et al. (36–38) in the developing sheep brain have shown that endothelial NOS expression and activity is greatest in most brain regions early in gestation, decreasing during maturation. Therefore, it is likely that the observed age-dependent effects of L-NAME on the arterial baroreflex reflect a greater distribution of endothelial NOS and/or neuronal NOS early in life in centers that normally modulate cardiovascular homeostasis. Further studies are warranted to confirm this.

In conscious normotensive Wistar rats, Minami et al. (32) assessed the effects of intravenous injection of 10 mg/kg of L-NAME on mean arterial pressure and pulse interval at 20 min. They observed that the arterial baroreflex was shifted toward a higher mean arterial pressure 20 min after L-NAME administration and that there was a significant increase in gain, with no effects on the HR range. The authors concluded that NO tonically inhibits the gain of the baroreceptor reflex, at least in the Wistar rat. These results should, however, be interpreted with caution in light of the aforementioned discussion regarding brain NOS activity after L-NAME administration. In conscious rabbits, Du et al. (12) investigated the role of endogenously produced NO in influencing the arterial baroreflex control of HR. They infused the L-arginine analog $N^G$-nitro-L-arginine (L-NNA; 15 mg/kg iv) over 30 to 40 min and assessed the arterial baroreflex 15 min later as well as at 24 and 48 h. L-NNA significantly reduced minimum HR at 15 min and 24 h, thereby increasing the HR range. There were no significant effects of L-NNA on the gain of the reflex. Fujisawa et al. (14) measured the effects of 10 mg/kg L-NAME on the arterial baroreflex control of HR in conscious rats; they showed that L-NAME decreased the HR range with no change in the gain of the reflex. In conscious rabbits, Liu et al. (27) measured the effects of an intravenous bolus injection of 13 mg/kg L-NNA on the arterial baroreflex control of HR 20 min after its administration. Minimum HR was significantly decreased after L-NNA, and there was an increase in the HR range. In a followup study, they measured the acute effects of intraperitoneal injection of the specific neuronal NOS inhibitor 7-nitroindazole on the arterial baroreflex control of HR in anesthetized as well as conscious rabbits (34). In conscious animals, 7-nitroindazole reduced minimum HR, although there was no effect on the gain of the arterial baroreflex. Taken together, these findings are in agreement with our observations of a decrease in HR range as well as a decrease in minimum HR after L-NAME administration to conscious 1-wk-old lambs, although we also observed a decrease in maximum gain after L-NAME administration to newborn lambs. Data obtained from studies in anesthetized rabbits by Jimbo et al. (24) are in agreement with our observations in 6-wk-old lambs. The arterial baroreflex control of HR was measured before and after administration of $N^G$-monomethyl-L-arginine (L-NMMA); no changes in any of the parameters of the arterial baroreflex were measured after L-NMMA. The authors concluded that NO does not modulate the arterial baroreflex in adult rabbits (24). Our finding that there was no apparent effect of NO in modulating the baroreflex control of HR in 6-wk-old lambs confirms their observations.

One possible limitation to our experimental design is the use of the NO donor sodium nitroprusside to lower arterial pressure to assess one limb of the arterial baroreceptor reflex. This choice was based on the previous observations that sodium nitroprusside fails to directly alter baroreceptor afferent activity, measured in anesthetized rabbits (30), or the arterial baroreceptor reflex itself, measured in conscious humans (21). In addition, previous studies investigating the effects of endogenously produced NO on the arterial baroreflex in conscious rats (14, 32) and rabbits (12, 27) used an experimental protocol of a combination of phenylephrine and sodium nitroprusside to alter arterial pressure. It is, however, well recognized that NO produced by administration of NO donors can elicit direct cardiac effects (25). For example, Schwarz et al. (39) showed that the NO donors S-nitroso-N-acetyl-dl-penicillamine and 3-morpholinosydnonimine (SIN-1) inhibit electrically evoked norepinephrine overflow from the rabbit Langendorff heart preparation, and studies (17, 18) on primary pacemaker cells of the rabbit heart have shown that the NO donor SIN-1 elicits cholinergic inhibition of $i$-type Ca$^{2+}$ currents through the generation of cGMP. Furthermore, in the isolated guinea pig atria, the NO donor sodium nitroprusside elicits a positive chronotropic effect (8, 35), although this effect develops much more slowly than the arterial baroreflex mediated increase in HR after its in vivo bolus injection (20). Therefore, it is unlikely that any direct cardiac effects of NO after administration of sodium nitroprusside or other NO donors would occur unless injected over several minutes and not as a bolus over seconds (6).
In conclusion, our experiments provide new information that there are age-dependent changes in the arterial baroreflex control of HR during postnatal development in conscious sheep. These maturational alterations in the arterial baroreflex appear to result from the effects of endogenously produced NO because they are abolished after administration of L-NAME but not t-NAME. The mechanism(s) underlying this age-dependent effect of NO is not known but may reflect age-dependent changes in central endothelial NOS and/or neuronal NOS distribution.

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