Regional Variation of Lamb Blood Vessel Responsiveness to Vasoactive Agents during Fetal Development

CHE SU, JOHN A. BEVAN, NICHOLAS S. ASSALI, AND CHARLES R. BRINKMAN, III

SUMMARY  Eight types of blood vessels were isolated from the fetal lamb and compared for their responsiveness to norepinephrine (NE), serotonin (5HT), and acetylcholine (ACh). Fetuses ranged from 53 days to term in gestational age, and from 0.05 to 4 kg in weight. The maximal contractile responses to the three agents were unequal among the vessels in the immature, premature, or mature periods of gestation. The vessels in the mature period were of three classes, in increasing order of maximal response to NE per unit cross-sectional area of vascular wall: (1) thoracic aorta, ductus arteriosus, and pulmonary artery; (2) common carotid, ulnar, and mesenteric arteries; and (3) renal artery and saphenous vein. 5HT and ACh were less consistent than NE in the magnitude of their effect and often elicited dilation or failed to cause a response in the immature and premature vessels. The vessels also were categorized according to gestation-related changes in their maximal responses. The responses to NE and 5HT of all vessels, corrected for the cross-sectional area, either remained unchanged or increased at different rates. The ductal contractile response to ACh decreased toward term. The aorta, pulmonary artery, and ductus arteriosus were different from one another in their affinity to NE, although no change in the affinity occurred with progress in fetal maturation. It is concluded that quantitative and qualitative diversity in the response to NE, 5HT, and ACh exists among blood vessels from early prenatal life.

REGIONAL variation in the responsiveness of blood vessels to vasoactive agents has been well established. Numerous examples of such variation in relation to biogenic amines, polypeptides, and prostaglandins have been cited.1 In addition to the vascular smooth muscle itself, the adrenergic and nonadrenergic motor innervation shows considerable qualitative and quantitative variation among different circulatory beds and consecutive and nonconsecutive vascular segments of the same bed.2 The nature, density, and pattern of innervation are factors that determine vascular responsiveness to drugs.3

Effects of various autonomic drugs on the lamb fetal circulation have been extensively investigated.4 Among the fetal blood vessels, the ductus arteriosus is unique for being highly responsive to acetylcholine (ACh). However, a systematic investigation of the fetal vascular segments from different circulatory regions is yet to be undertaken.

The purpose of the present work was to determine whether fetal blood vessels are heterogeneous in their affinity for or maximal response to neurohumoral agents and, if so, whether such heterogeneity changes with gestational development.

Methods

Twenty-three ewes with dated pregnancies were used. Following spinal anesthesia with tetracaine, the fetus was delivered rapidly by cesarian section. The fetal head was covered immediately with a saline-filled rubber glove to prevent breathing. Under local anesthesia with lidocaine,
Results

Results are grouped in relation to three periods of fetal maturity: premature (period I), immature (II), and mature (III). The maximal contractile responses to vasoactive agents are an important parameter of smooth muscle reactivity. Because the thickness of vascular wall that contains smooth muscle differs greatly, depending on the vessels and the gestational age, it was necessary to standardize the responses to facilitate comparisons. As a first approximation, the responses were divided by the cross-sectional area of the vascular wall. The maximal responses of the vessels to NE, 5HT, and ACh are illustrated in Figure 1.

The analysis of variance shows that the mean maximal responses of different vessels to NE are unequal at the 5% level of significance. This is true also of the mean maximal responses to 5HT and for NE or 5HT, within any of the three gestational periods. For ACh, only results from the period III vessels were appropriate for analysis, and they also indicate that the vessels are unequal in their maximal responses. Although all ductus arteriosus preparations contracted to ACh, many other types of vessels from periods I and II showed wide variations. Within period I, pulmonary arteries were taken from eight fetuses; of these, four failed to respond to ACh. Responses of three ulnar artery preparations ranged from nil to over 1 kdyn/mm². Five other types of vessels were practically unresponsive to ACh. Within period II, all eight pulmonary artery preparations tested contracted to ACh. With other kinds of vessels, half or more of the tested preparations were totally unresponsive while the remainder contracted as much as 0.8 kdyn/mm² and, hence, an estimate of the mean or analysis of variances was unwarranted.

Multiple comparisons between the mean maximal responses were performed to identify the individual differences. On the basis of confidence limits that suggest a significant difference between two means, the vessels must be considered to be highly heterogeneous. Thus, for the mature fetuses, the responses of the thoracic aorta, ductus arteriosus, or pulmonary artery were significantly smaller than those of the carotid, ulnar, or renal artery, or saphenous vein. For convenience these vessels and the mesenteric artery will be abbreviated to A, D, P, C, U.
R, S, and M, respectively. Responses of C were smaller than those of R, and M smaller than R or S. Numerous other comparisons will not be described but it is noteworthy that A-D-P as a group differed from any other individual vessel, from C-U-M as a group, and from R-S as a group. Finally, response of the group C-U-M was smaller than that of R-S combined as a group. It may be restated that the eight types of vessels from mature fetuses can be divided into three classes consisting of A-D-P, C-U-M, and R-S, in increasing order of maximal response to NE.

A similar pattern was found among vessels of the premature fetuses. The maximal NE-induced responses of A, D, and P, individually or as a group, were significantly smaller than those of U, M, and R, individually or as a group. C and S gave intermediate responses not significantly different from those of other vessels. Among vessels of immature fetuses, apart from the fact that the ulnar artery was significantly greater than any other vessel in its maximal response to NE, D-P-C as a group was lower than M-R or A-M-R-S in this parameter. Response of M, R, and S may be greater than that of other vessels except for the ulnar artery, but no significant differences could be established due in part to the small number of observations (see Fig. 1).

For another vasoactive agent, 5HT, among vessels of period III, response of A, D, or P was significantly different from that of C, R, or S, and S was significantly different from M. Just as with NE, A-D-P, C-U-M, and R-S as groups were in order of increasing response. These descriptions applied in general also to vessels of period II, although responses of C-U-M and R-S were comparable. Relatively large variance was associated with period I vessels whereas the response of the ulnar artery was significantly greater (1.94 ± 0.85 kdyn/mm²) than that of any other vessel by comparisons based on F statistics; a paired t-test indicated that this artery was significantly different only from A, D, or P at 5% level.

It is not apparent from the above analysis or Figure 1 that 5HT was less consistent in effect than NE, which invariably induced a contractile response of all vessel preparations. For example, 5HT was without effect on three of nine aortic preparations, and four of eight pulmonary arteries, of period I. The coefficient of variation of mean maximal response to 5HT was higher than that to NE for all types of vessels except for the carotid artery. Furthermore, whereas all ductus arteriosus preparations of period I contracted to NE and ACh, four relaxed by 0.04–0.19 kdyn/mm² in response to 5HT; in addition, three preparations contracted and two were totally unresponsive to 5HT (Fig. 2).

The consistency with which ACh induced a contractile response of vessels of period III was relatively greater than for vessels of the earlier periods. Saphenous vein was greater in this response than A, P, C, U, and M. Vessels of periods I and II, except for the ductus arteriosus, responded much less consistently to ACh so that comparisons among these vessels were impractical.

In the above description, comparisons were made primarily among the types of vessels within a given gestational period. Figure 1 also illustrates the gestation-related changes in vascular reactivity. By analysis of variance, the mean maximal responses of C, R, or S were unequal among the three periods with respect to either NE or 5HT. The procedure of multiple comparisons showed that the mean of period III was significantly greater than that of period I for any of these three vessels and for NE or 5HT. No such significant changes could be identified with other vessels. As can be seen from Figure 1, no gestation-related change in responses to NE or 5HT was likely for the pulmonary artery and ductus arteriosus. The ductus arteriosus was unique in that the response to ACh appeared to decrease toward term. The mean responses of the three periods were indeed unequal; namely, the mean response of period III was significantly smaller than that of period I or II.

In the present work, the stress (or basal stretch per unit cross-sectional area of vascular wall) applied to vessels was dependent on the size of vessels, since a uniform 1-g passive stretch was applied to all preparations. As this factor might contribute to the observed variability of responses, the relationship between the stress on the vascular wall and the maximal responses was examined. Analysis of variance indicated that the mean stresses on the eight vessels were unequal within period I, II, or III. The three large elastic arteries, A, D, and P, as a group, were larger in cross-sectional area than the other five vessels and hence bore a smaller stress. However, the mean stresses were not significantly different among the latter five vessels. When a correlation between the mean stress and mean NE maximal responses of these five vessels was sought, low correlation coefficients, 0.05, 0.44, and 0.17 for the period I, II, and III, respectively, were obtained. Thus, no causal relationship could be detected between the stress and the magnitude of response among the five vessels.

An essential characteristic of reactivity of a smooth muscle is the affinity of its receptors for drugs, estimated by the median effective dose (ED₅₀) in this study. The ED₅₀ values for NE in relation to the three largest arteries, A, D, and P, are presented in Table 1; data were insufficient for other agents or vessels. The NE ED₅₀ of any artery did not change significantly with gestation. The means among these arteries are unequal within any of the three gestational periods (Table 2). The ED₅₀ of P
was always significantly greater than that of A or D or A-D as a group (Table 3).

**Discussion**

This work has demonstrated that fetal blood vessels from the time of early pregnancy constrict or dilate to the vasoactive agents NE and 5HT and, to a lesser extent, to ACh. This is in keeping with the finding that there is autonomic nervous control of circulation in vivo in lamb fetuses of the premature and mature periods as reported by several investigators. The present work in vitro has extended the period of observation to the immature fetus, as young as 53 days in gestational age and as small as 50 g in body weight.

Because of the limited amount of vascular tissue obtainable from fetuses and the lack of previous investigation of this problem, the present work was essentially a survey. Mechanisms for inactivation of NE, 5HT, and ACh such as tissue uptake and enzymatic degradation may influence the magnitude of the responses. Our previous study on the common carotid artery of the lamb fetus showed that functional adrenergic innervation lags behind the vascular smooth muscle response to NE, the transmitter. Therefore, the neuronal uptake of NE may play a limited role in the inactivation of exogenous NE, at least in the immature fetuses.

The unequal maximal responses to agonists cannot be ascribed to nonuniformity of stress applied to the vascular preparations. Among five vessels for which the stresses were not significantly different, the maximal responses to NE were unequal within period III (mature fetuses): the maximal renal artery response was greater than that of the renal artery and saphenous vein. Significant differences in responses to 5HT and ACh among individual vessels or groups of vessels also were demonstrated. Furthermore, qualitative differences were seen in the ED50 to NE among the aorta, pulmonary artery, and ductus arteriosus. There is no reason to believe that the ED50 is affected appreciably by varying the stress. The stresses were in fact not significantly different among these three arteries.

The magnitudes of responses in this study were expressed in relation to cross-sectional area of vascular wall in an attempt to correct for differences in mass of the contractile elements between different types of vessels and between different gestational stages. For this reason, the gestation-related increase in responses of the more muscular vessels probably is not attributable to thickening of the wall but rather to a change in smooth muscle cells themselves. The wall of elastic-type arteries would be expected to contain a smaller proportion of smooth muscle than the muscular-type vessels. This may account in part for the fact that responses of the aorta, pulmonary artery, and ductus arteriosus generally were smaller than those of the other more muscular vessels.

**Table 1**  
**Norepinephrine ED50 (μM) of Fetal Aorta, Pulmonary Artery, and Ductus Arteriosus**

<table>
<thead>
<tr>
<th>Period</th>
<th>Aorta Mean</th>
<th>CI*</th>
<th>n</th>
<th>Ductus arteriosus Mean</th>
<th>CI</th>
<th>n</th>
<th>Pulmonary artery Mean</th>
<th>CI*</th>
<th>n</th>
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<tr>
<td>I</td>
<td>3.3</td>
<td>1.3-8.8</td>
<td>9</td>
<td>1.4</td>
<td>0.7-2.9</td>
<td>7</td>
<td>10.1</td>
<td>4.1-25.0</td>
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<tr>
<td>II</td>
<td>5.4</td>
<td>4.0-7.2</td>
<td>5</td>
<td>0.8</td>
<td>0.2-4.0</td>
<td>4</td>
<td>14.0</td>
<td>10.3-18.8</td>
<td>4</td>
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<td>III</td>
<td>4.0</td>
<td>2.5-6.4</td>
<td>6</td>
<td>1.6</td>
<td>0.3-8.2</td>
<td>6</td>
<td>13.1</td>
<td>11.8-14.5</td>
<td>6</td>
</tr>
</tbody>
</table>

* CI = 95% confidence intervals.

The unequal maximal responses to agonists cannot be ascribed to nonuniformity of stress applied to the vascular preparations. Among five vessels for which the stresses were not significantly different, the maximal responses to NE were unequal within period III (mature fetuses): the maximal renal artery response was greater than that of the carotid or mesenteric artery, and the maximal saphenous vein response was greater than that of the mesenteric artery. The ulnar, carotid, and mesenteric arteries as a group had a lesser maximal response than the renal artery and saphenous vein. Significant differences in responses to 5HT and ACh among individual vessels or groups of vessels also were demonstrated. Furthermore, qualitative differences were seen in the ED50 to NE among the aorta, pulmonary artery, and ductus arteriosus. There is no reason to believe that the ED50 is affected appreciably by varying the stress. The stresses were in fact not significantly different among these three arteries.

**Table 2**  
**Analysis of Variance of Norepinephrine ED50 among Fetal Aorta, Pulmonary Artery, and Ductus Arteriosus**

<table>
<thead>
<tr>
<th>Period</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
<th>F ratio</th>
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<tr>
<td>I</td>
<td>Means</td>
<td>581</td>
<td>2</td>
<td>17.1</td>
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<tr>
<td></td>
<td>Within</td>
<td>892</td>
<td>21</td>
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<tr>
<td></td>
<td>Total</td>
<td>1473</td>
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</tr>
<tr>
<td>II</td>
<td>Means</td>
<td>323</td>
<td>2</td>
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<tr>
<td></td>
<td>Within</td>
<td>103</td>
<td>10</td>
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<tr>
<td></td>
<td>Total</td>
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<td>12</td>
<td></td>
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<tr>
<td>III</td>
<td>Means</td>
<td>342</td>
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<td></td>
<td>Within</td>
<td>93</td>
<td>15</td>
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<tr>
<td></td>
<td>Total</td>
<td>435</td>
<td>17</td>
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</table>
The muscular ulnar artery does not show a gestational-related changing trend but the data are limited.

The ductus arteriosus is a unique structure in that its muscular arteries, including the carotid, renal, and possibly mesenteric, initially gain reactivity rapidly and then the response levels off toward term. The response of the saphenous vein alone appears to increase through term. The muscular ulnar artery does not show a gestational-related changing trend but the data are limited.

The ductus arteriosus is a unique structure in that its maximal contractile response to ACh remains relatively high and surpasses that of other vessels during the two earlier periods but declines during the last period. ACh elicited a somewhat greater constriction than NE in the ductus preparations from the immature and premature lamb fetuses but not in those from the mature fetuses. It is necessary, therefore, to make a distinction with respect to gestational age when describing drug effects on this vessel. Such a decline in reactivity to ACh toward term does not favor the possibility that ACh-induced constriction may participate in closure of the ductus.

Since the saphenous vein was comparable to the four arteries in many parameters, differences in the transmural pressure in vivo and hence in the long-term stress on vascular wall are unlikely to be major determinants of the responsiveness of smooth muscle cells. In view of the finding that the maximal responses to adrenergic and nonadrenergic agents (NE and 5HT) were roughly parallel among different vascular species throughout gestation, the variation does not appear to be the consequence of any specific trophic influence of the adrenergic nerves.

In sum, it is evident that the blood vessels of the lamb fetus diversify, or differentiate, from early gestation. This is manifest not only quantitatively with respect to the relative maximal responses to vasoactive agents at a given gestational age and the gestation-related changes of these responses, but qualitatively in the drug-receptor affinity and the nature of responses (relaxation-contraction) to a given agent.

References

### Table 3

<table>
<thead>
<tr>
<th>Period</th>
<th>Aorta</th>
<th>Ductus arteriosus</th>
<th>Pulmonary artery</th>
<th>L</th>
<th>Confidence limits</th>
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<td></td>
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<tr>
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<td>3.38</td>
<td>-0.25</td>
<td>6.51</td>
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<td></td>
<td>3.26</td>
<td>5.46</td>
<td>11.98</td>
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<tr>
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<td>[-1/2]</td>
<td></td>
<td>3.48</td>
<td>11.37</td>
<td>18.33</td>
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<tr>
<td>II</td>
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<td>-1</td>
<td>3.44</td>
<td>-0.07</td>
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<td></td>
<td>2.48</td>
<td>7.19</td>
<td>12.15</td>
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</table>

NE and 5HT throughout the immature (I), premature (II), and mature (III) periods. The more peripheral, more muscular arteries, including the carotid, renal, and possibly mesenteric, initially gain reactivity rapidly and then the response levels off toward term. The response of the saphenous vein alone appears to increase through term. The muscular ulnar artery does not show a gestational-related changing trend but the data are limited.

**Figure 3** Schematic illustration of developmental patterns of responses of fetal vascular smooth muscle. Ordinate: maximal responses to norepinephrine and serotonin, except that (D) denotes maximal response to acetylcholine. Abscissa: gestational period. The abbreviations are the same as in Figure 1. See text for detail.
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