Development of Baroreflex Activity in Unanesthetized Fetal and Neonatal Lambs

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ABSTRACT

Baroreflex activity was assessed in 10 fetal lambs at 85–145 days of gestation. ECG leads sewn to the fetal chest wall, vinyl catheters in brachial artery and femoral vein, and a balloon catheter in the descending aorta were exteriorized through the ewe’s flank. After at least 36 hours, the reflex bradycardia, in response to increased blood pressure by balloon inflation, was measured. Baroreflex sensitivity was expressed as the slope of the beat-to-beat relationship between systolic (S.P.) or pulse (P.P.) pressure and the subsequent R-R interval or instantaneous heart rate (H.R.). Baroreflex activity was considered absent if the slope did not differ significantly from zero or if the correlation coefficient was less than 0.7. Throughout gestation baroreceptors could respond to pressure elevation, but the proportion of positive responses increased with age. Baroreflex sensitivity increased up to term when either S.P. or P.P. were plotted against the next R-R interval. Regression analysis of S.P. or P.P. vs. R-R interval, or of P.P. vs. H.R showed increasing baroreflex sensitivity with maturation Analysis of S.P. vs. H.R. showed no significant increase in response with advancing gestation; however, this type of analysis does not take into account the slower resting heart rates of older animals.

KEY WORDS

Autonomic blocking agents autonomic nervous system blood pressure carotid sinus electrocardiogram heart rate fetal maturation gestation mechanoreceptors vagus nerve

The baroreceptor reflex is a well-known compensatory mechanism for buffering sudden changes in systemic blood pressure in adult animals and man. It is also possible that this baroreflex might participate in the abrupt adaptation that occurs at birth to balance a decreased pulmonary vascular resistance, an increased systemic vascular resistance and a variable blood volume. Although other workers have shown that baroreflex responses could be elicited in fetal (1–4) and newborn (1) animals, all previous studies have been conducted in anesthetized animals with the fetus exteriorized. Since it is now well known that cardiovascular function in the fetus may be altered by anesthesia or exteriorization (5), we studied fetal lambs in utero while the unsedated ewe stood quietly. The reflex cardiac slowing in response to rapid elevation of blood pressure could then be measured throughout the latter half of gestation.

Methods

Ten time-dated pregnant ewes (Dorset or Southdown breeds) with fetuses of gestational ages ranging from 85 to 145 days and six neonatal lambs were studied. Food was withheld for 24 hours prior to surgery, and spinal anesthesia was administered to the ewes by intrathecal injection of 2.0 ml of 1% tetracaine hydrochloride (Pontocaine). After insertion of a catheter into the maternal femoral artery, the uterus was exposed...
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by a midline abdominal incision. Vinyl catheters (0.030-inch i.d., 0.048-inch o.d.) were inserted into a fetal femoral and brachial or carotid artery and into a saphenous vein (6). A balloon catheter (nos. 3 to 5 French, maximum inflated diameter of 9-12 mm) was inserted into the other fetal femoral artery and was advanced into the descending aorta between the renal arteries and flank. The catheters were filled with heparin solution (1000 U.S.P. units/ml) and capped with metal plugs.

Not less than 36 hours later, when the ewe had recovered from surgery, experiments were carried out while the animal was standing quietly with no medication. Only fetuses with a stable heart rate and normal blood gases (carotid arterial Po2 > 20 mm Hg, Pco2 < 45 mm Hg and pH > 7.32) (6) were included in the study.

Fetal arterial pressures were monitored using Statham P23Dc pressure transducers positioned at a miduterine level and, together with the electrocardiogram, were recorded both onto a direct writing recorder (Beckman Type R Dynograph) and a magnetic tape recorder (Ampex) running at 15 inches/sec. By replaying the tape at 1½ inches/sec and running the paper on the direct recorder at 100 mm/sec, the time scale could be expanded and the R-R interval on the electrocardiogram measured to 0.5-msec accuracy. The amplitude response of the catheter transducer system was uniform to 12 Hz as determined by the pressure transient method (7). In some experiments instantaneous heart rate was recorded by a Beckman cardiotachometer directly from the arterial pressure wave form.

When the balloon catheter was quickly inflated with saline, there was an elevation of blood pressure in the arterial circuit above the balloon associated with an immediate cardiac slowing (Fig. 1). Because the balloon either completely occluded the aorta or severely restricted flow, the pressure in the descending aorta was lowered and umbilical arterial and placental flow were probably compromised. Subsequent changes in fetal blood gases and pH could influence chemoreceptors and might modify the cardiac slowing associated with balloon inflation. We therefore carried out experiments early in the study to ascertain how best to avoid the influence of such changes. Blood gas measurements were made on samples withdrawn from the brachial artery at approximately 5, 10, 15 and 20 seconds after balloon inflation.

No blood gas changes were detected in the fetal arterial blood before 5 seconds, although from this time onward Pco2 rose while Po2 and pH fell. Accordingly, in quantifying the cardiac slowing associated with balloon inflation, measurements were made for not more than 5 beats of increasing pressure. At initial heart rates of 150 to 200/min the time taken for 5 beats was less than 3 seconds, well before blood gases would alter.

Evidence that the cardiac slowing associated with the acute elevation of blood pressure in the cephalic part of the fetus was indeed mediated by baroreceptors was afforded by the following acute experiment on a fetus of 128 days' gestation (Fig. 2). Catheters and electrocardiographic leads were positioned as usual and the fetus returned to the uterus, where it remained during all subsequent measurements. Balloon inflation was associated with an elevation of proximal arterial blood pressure and a rather marked cardiac slowing. The inflation was repeated several minutes after the acute section of both carotid sinus nerves, and the heart rate response was noted to be diminished but still present. The aortic arch was then denervated by stripping the adventitia from the ascending aorta to the junction with the ductus arteriosus, the chest closed, and the fetus again returned to the uterus. Thereafter, balloon inflation was not associated with any demonstrable cardiac slowing. Electrical stimulation of the peripheral end of the right vagus nerve still produced bradycardia, indicating intact vagal afferents despite arch denervation.

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To examine whether the observed cardiac slowing associated with balloon inflation was related to changes in either the parasympathetic or sympathetic influences or cardiac pacemaker cells, experiments were performed in which responses to balloon inflation were studied before and after the administration of autonomic blocking agents. These drugs were administered into the fetal saphenous vein in a volume of 2 ml (including flush) over a period of 1 minute. Beta-receptor blockade with propranolol was produced in 30 experiments over all gestational ages studied. Sufficient propranolol was administered (1 mg/kg) to block the tachycardia associated with the intravenous injection of a test bolus of isoproterenol (0.05 μg/kg) and the balloon inflation was repeated about 10 minutes after the injection of the blocking agent. Parasympathetic blockade with atropine was produced in 60 experiments over all gestational ages studied. Sufficient atropine was administered (200 μg/kg) to block the transient bradycardia associated with the intravenous injection of a test bolus of acetylcholine (15 μg/kg). All drug doses were based on fetal weights estimated for each gestational age (8).

Balloon inflation was repeated not more than five times on a particular day to avoid weighting of data by repeated observations on the same animal. Individual animals were studied sequentially over the following periods of gestational days for only as long as the arterial blood gases remained normal: 85–107, 88–98, 98–107, 98–104, 107–113, 111–118, 113–134, 117–135, 137–145, 137–144. The last three lambs were also studied after delivery. Three ewes spontaneously delivered their lambs with catheters attached and baroreflex activity was studied in these healthy,
conscious neonates to investigate whether baroreflex sensitivity changed abruptly at birth.

The baroreflex response was quantified by a method similar to that used by Smyth et al (9) and Bristow et al. (10) in adult man but modified for the fetal lamb. As pressure rose, the R-R interval increased. For the 8 beats analyzed (3 control and the first 5 during balloon inflation), the systolic or pulse pressure was plotted against the pulse interval of the next beat, measured as R-R interval on the electrocardiogram. Using the method of least squares a rectilinear regression was calculated for the relationship between pressure and R-R interval. The slope of this line, or regression coefficient, then gave an estimate of baroreflex activity. The latency of the reflex heart rate response to increments of pressure in fetal lambs of different stages of gestation is not known. We therefore carried out three sets of regression analyses for each balloon inflation. In the first analysis, the systolic blood pressure of each of the 8 beats was correlated with the first subsequent R-R interval, i.e., the one immediately following each beat. In the second analysis, however, each of the same 8 beats was correlated with the second R-R interval following each beat, and in the last analysis, each of the same 8 beats was correlated with the third subsequent R-R interval.

Regression coefficients were also calculated for systolic and pulse pressure against the instantaneous heart rate of the next beat. This is numerically related to pressure vs. the reciprocal of the R-R interval. Despite the hyperbolic relationship of instantaneous heart rate per minute to R-R interval, the correlation coefficients for rectilinear regression of both types of analysis were comparable.

Baroreflex activity was considered to be effectively absent if the value of the regression coefficient did not differ significantly from zero at the 0.01 probability level or if the correlation coefficient was less than 0.7.

The fetuses were divided into three groups according to days of gestation: under 100 days, 101-120 days, and 121 days to term. In the statistical analysis of change in reflex sensitivity with maturation, the data were treated as a continuous function of gestational age.

**Results**

When the balloon was inflated in the fetal descending aorta, no changes were detected in maternal blood pressure, heart rate, or blood gases, thus excluding the possibility that maternal factors influenced the acute fetal baroreflex response.

When the response was analyzed by plotting beat-to-beat blood pressure against the first, second, and third R-R interval, we found that, throughout the gestational periods studied, plots of systolic pressure vs. the first subsequent R-R interval most consistently approached linearity and that the correlation coefficients for linear regression analysis of these were highest.

As shown in Figure 3, the cardiac slowing associated with balloon inflation could be completely abolished by parasympathetic...
Baroreflex responses in fetal and newborn lambs. Histograms show the increase in the proportion of positive responses with advancing gestational age when the baroreflex was expressed as the rectilinear regression coefficient of (a) systolic pressure vs. R-R interval ($P < 0.005$); (b) systolic pressure vs. instantaneous heart rate ($P < 0.005$); (c) pulse pressure vs. R-R interval ($P < 0.005$); (d) pulse pressure vs. instantaneous heart rate ($P < 0.025$). The statistical analysis was by the Chi-square test and excludes the neonatal lambs, $n =$ total number of observations in the group.

Evidence of Baroreflex Activity during Gestation.—We considered baroreflex activity to be manifest when the regression coefficient differed significantly from zero at the 0.01 probability level and the correlation coefficient was greater than 0.7. This is arbitrary; however, it was apparent that on any one day, even with a stable heart rate and normal blood gases, there was within-animal variability in the presence or magnitude of significant reflex cardiac slowing. At all gestational ages studied, baroreflex responses were elicited (Fig. 4). Whether the analysis was of pressure vs. R-R interval or heart rate, the proportion of positive responses increased with gestational age, although in the newborn lambs the proportion of positive responses was less.

The magnitude or sensitivity of the baroreflex with increasing gestational age is plotted in Figures 5 and 6 and the levels of significance of the change with gestational age are shown in Table I, where the data are treated as a continuous function and the animals are not divided into groups. Plots of systolic pressure against the first subsequent R-R interval (Fig. 5) show a highly significant increase in the magnitude of response with maturation. When systolic pressure was plotted against instantaneous heart rate (Fig. 5), however, less tendency for increasing sensitivity during gestation was detected, although the neonatal lambs had greater responses.

When the regression coefficients of pulse pressure vs. R-R interval or instantaneous rate (Fig. 6) were plotted, the magnitude of response increased significantly with gestational age. Significant differences were also detected between the different groups of fetal and neonatal lambs.

### TABLE 1

<table>
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<th>Regression line $y = bx + c$</th>
<th>$s_0$ of slope $b$</th>
<th>$t_b$</th>
<th>$d.f.$</th>
<th>$P$</th>
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<td>S.P. vs. R-R</td>
<td>$y = 0.057x - 5.041$</td>
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<td>$y = -0.006x - 3.19$</td>
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<td>2.559</td>
<td>113</td>
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<td>P.P. vs. R-R</td>
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<td>6.249</td>
<td>95</td>
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<tr>
<td>P.P. vs. H.R.</td>
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<td>3.598</td>
<td>98</td>
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</table>

* S.P. = systolic pressure; H.R. = heart rate; P.P. = pulse pressure.
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In the animals studied 1 or 2 days before delivery and then the day after spontaneous delivery, no significant increase in sensitivity was found between the pre- and postnatal responses.

Discussion

Alterations in heart rate are but one component of the baroreflex response. Arterial and venous dilatation as well as change in force of cardiac contraction contribute to the cardiovascular response to increase in blood pressure. It was not the purpose of this study to evaluate the various components of the reflex in the fetal lamb but to investigate when baroreflex activity could first be detected, to quantify the sensitivity of the reflex and to document the alteration in sensitivity throughout the latter half of gestation.

No previous quantitative assessment of baroreflex activity has been carried out in intact fetuses in utero, nor have serial studies been attempted during gestation. Barcroft and Barron (1) detected acute bradycardia in response to injection of epinephrine or norepinephrine and found that it was abolished by vagal section. This was confirmed by Dawes et al. (2) in fetuses as young as 90 days. In animals near term, acute carotid occlusion causes a rise of arterial pressure, and impulses with the same periodicity as the heart rate can be detected in the afferent nerve from the carotid sinus (4, 11). Brinkman et al. (3) presented evidence for reflex changes in peripheral vascular resistance in exteriorized fetuses of “near-term” sheep and also detected bradycardia in response to elevation of pressure in an isolated carotid sinus pouch.

The technique used to quantify baroreflex sensitivity in this study was similar to that used by Sleight’s group (9, 10). The means of achieving rapid transient elevation of blood pressure, however, has been modified for the fetal lamb preparation. Instead of the bolus injection of pressor agents such as angiotensin or phenylephrine, blood pressure was elevated by inflation of a balloon in the descending aorta. In addition to the lack of possible central actions, balloon inflation has other theoretical advantages over the use of pharmacologic pressor agents. This means of stimulation would not alter the elastic modulus of the vessel wall. Aars (12) has shown that alpha-receptor-stimulating agents may alter the stress-strain characteristics of the carotid sinus wall, which would in turn modify baroreceptor sensitivity. Because the fetus is subject to increasing autonomic drive during gestation (13), sympathomimetic
agents or angiotensin could alter the characteristics of the vessel wall to a different extent, depending on fetal maturity.

Balloon inflation occludes the descending aorta and hence reduces umbilical blood flow. When prolonged, this is accompanied by a fall in pH and Po_{2}, rise in Pco_{2}, and probable release of catecholamines associated with the asphyxia (14). Accordingly, baroreflex activity was assessed during not more than the first 3 seconds of balloon inflation. This was before changes in brachial or carotid arterial blood gases could be detected and probably before release of catecholamines could influence the response. Observations were made only when the unrestrained ewe was standing quietly with stable blood pressure and heart rate and normal blood gases. In contrast to previous acute studies on exteriorized fetuses, the likelihood of fetal reflex activity being influenced by abnormal maternal catecholamine levels, anesthesia, compromised placental blood flow, or abnormal uterine movements was minimized.

The proportion of positive baroreflex responses increases with advancing gestational age although the neonatal lambs respond less consistently than late-gestation fetuses. This paradoxical finding might be due to the influence of respiration in the neonatal lambs. All the responses of the neonatal lambs were analyzed, and balloon inflation was not timed to coincide with a constant phase of respiration but, as has been previously shown in adult man (10), if expiration coincides with baroreceptor stimulation, the reflex bradycardia in response to elevation of pressure will be reduced or abolished.

Although at first it might seem superfluous to analyze the relationship of blood pressure to both R-R interval and instantaneous heart rate, the numerical result of the latter is dependent on the initial heart rate. For example, if for a rise of 1 mm Hg in pressure, the heart rate falls by 2 beats/min from an initial rate of 100 beats/min, representing a 2% change in time interval between beats, the R-R interval would now increase by 12 msec. If these trends continued as pressure rose further, the regression coefficients calculated for pressure vs. heart rate would be the same in both situations, whereas regression coefficients for pressure vs. R-R interval would be 3 msec/mm Hg in the first instance and 12 msec/mm Hg in the second. In the latter analysis, "baroreflex sensitivity" would appear to be four times that when the initial heart rate was 200/min. Apart from any question as to which type of analysis is more appropriate as an index of reflex sensitivity, the purely mathematical sequelae of comparing baroreflexes elicited at different initial heart rates must be taken into account. It has recently been argued (10) that comparisons of baroreflex sensitivity expressed as the regression coefficient of pressure vs. heart rate are invalid on the assumption that the relationship of blood pressure to heart rate was hyperbolic. No evidence, however, was given to support the notion that this relationship was in fact hyperbolic during the time that pressure was rising. Baroreflex sensitivity expressed as the regression coefficient of pressure vs. R-R interval favored by Bristow et al. (10) also has the major disadvantage of not taking into account initial heart rate. Each type of analysis has disadvantages but, rather than make an arbitrary selection, we have expressed our results both ways.

Resting heart rate decreases during gestation (6) and this possibly explains why regression coefficients of systolic pressure vs. R-R interval but not vs. heart rate show a greater and more significant increase with fetal maturation. When, however, the change in baroreflex sensitivity with maturation was expressed as the regression coefficient of pulse pressure vs. R-R interval or vs. instantaneous rate, both showed significantly increased sensitivity with gestation, the tendency being greater than in the analyses using systolic pressure.
Before discussing the significance of the change in magnitude of response with gestational age, it should be noted that since the ductus arteriosus is patent in the fetus, balloon inflation in the descending aorta elevates pressure in the pulmonary artery as well as in the systemic circuit. Thus, pulmonary arterial baroreflex activity (15) might contribute to the response. Preliminary experiments with an inflatable balloon catheter positioned at fetal thoracotomy so as to encircle the aortic isthmus have shown essentially similar results to those obtained with balloon inflation in the descending aorta. In the former, the pressure in the pulmonary artery was not immediately elevated when the aortic isthmus was abruptly narrowed. This would imply that pulmonary baroreceptor activity and a major change in systemic venous return did not play a significant part in our observed responses.

Baroreceptors are distortion receptors (16) that can be expected to respond to changes in systolic, diastolic, mean and pulse pressure, as well as to change in rate of rise of pressure or to combinations of the above. As gestation progresses, the dimensions of the aorta and carotid sinus region increase. If the baroreceptors were pressure receptors, then from Laplace’s law, for the same intra-arterial pressure the tension in the vessel wall would be greater as its circumference increased in the later stages of gestation. Increments in arterial pressure would similarly be associated with greater changes in wall tension and could thus have accounted for increasing baroreflex sensitivity with gestational age (higher regression coefficients). Baroreceptors, however, are distortion receptors and assuming no change (or a decrease [17]) in vessel wall compliance, the expected circumferential change for given increments of distending pressure will be smaller, not greater, in the larger vessels. Thus, if the change with age of baroreflex sensitivity were a function simply of vessel geometry, the smaller (less mature) rather than the larger fetuses would have had higher regression coefficients.

The question next arises at which level of the baroreflex arc structural and functional changes are occurring which underlie the increasing sensitivity with fetal maturation. The afferent receptors in the arterial wall may not be fully developed or the mechanical stress-strain properties of the vessel (i.e., elastin collagen ratio [18], salt-water content, etc.) may change with age or with variations in the level of circulating catecholamines (12). It should be pointed out, however, that in the absence of single-fiber recordings from baroreceptor afferent nerves, our findings do not necessarily relate to maturation of baroreceptor activity, since the central or efferent limbs of the reflex arc may be less mature at a time when the receptors are fully responsive. Throughout the period of gestation studied, we found the highest correlation coefficients for the relationship of pressure to rate (or R-R interval) when beat-to-beat blood pressure was correlated with the first subsequent rather than later beats (or R-R intervals). Although the time from the peak systolic pressure to the next P wave was much greater than the reflex time recorded for single fibers in the anesthetized dog (19), no information is available for conduction time and latency in the fetal lamb. Since the highest correlation coefficients were found between pressure and the first subsequent time interval at all periods of gestation studied, it is unlikely that altered latency (e.g., secondary to increasing nerve conduction velocity) would influence our findings.

The gain of the system might increase with fetal maturation when the number of afferent impulses in the buffer nerves increase for a certain baroreceptor distortion. Similarly, processing in the central nervous system may change and bring about an enhanced efferent discharge for a particular afferent input. The response of the heart to the same quantity of released neurohumoral transmitter, acetylcholine, could change, although other work from our laboratory has shown no evidence for this. Still another possibility is that more acetylcholine may be released from the vagus following comparable neuronal efferent impulse.

We have shown that baroreflex activity is present as early as 85 days of gestation in fetal lambs and that the sensitivity of the reflex
increases up to term. Whether the observed alterations depend on increasing receptor sensitivity, changes in afferent gain in the system, or maturation of cerebral function, remains to be determined.

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