Is baroreflex control of sympathetic activity and heart rate active in the preterm fetal sheep?

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METHODS

HYPOTENSION AND MARKED INSTABILITY of blood pressure occur in many premature infants, especially in the first few days of life; the underlying mechanisms are not completely understood (6). In adult life, the arterial baroreflex is essential in the short-term control of blood pressure and has two main efferent pathways: heart rate (HR), controlled by both the parasympathetic and sympathetic nervous system, and vascular resistance mediated by sympathetic nerve activity. Thus, potentially, immaturity of this key reflex system could contribute to the morbidity of premature birth (1).

Overall, studies in human neonate, mainly of HR changes in response to spontaneous changes in blood pressure or small changes using tilt-tables over short periods of time, are consistent with reduced sensitivity of the baroreflex with younger postconceptional age (1, 14, 18, 28). Animal studies investigating the immediate effects of blood pressure changes on HR have variably shown decreased baroreflex sensitivity in the preterm fetus compared with later in gestation (42) or no significant change in sensitivity with increased gestation (16, 26). One study found that there was no change in the magnitude of the increase in HR in response to mild hypotension over the past 35 days of gestation (49), but others have suggested that the afferent baroreceptor signal was actually more sensitive early in gestation (8), with apparently higher baroreflex sensitivity in the preterm fetus compared with closer to term (32).

Although the baroreflex control of heart rate is an important indicator of sympathetic and vagal control over the heart, it does not reflect sympathetic outflow to the vasculature, which plays a critical role in the regulation of peripheral resistance (24). Data from late-gestation fetal sheep suggest that directly measured renal sympathetic nerve activity (RSNA) in the near-term fetal sheep is under baroreflex control across an appropriate blood pressure range for the stage of gestation (30, 38–40, 43). Recently, we have shown that the preterm fetal sheep has discharges of RSNA coordinated with the cardiac cycle, although at a much lower frequency than in adults (9). This is generally considered to reflect the entrainment of inherently generated bursts of activity by pulsatile afferent baroreceptor input (3, 45). However, evidence suggests that entrainment can occur independently of baroreflex control of the mean level of RSNA (27). Thus, although reflex control of sympathetic activity is a key controlling homeostatic mechanism later in life, it is unknown whether RSNA responds to rapid changes in blood pressure in the preterm fetus. Further, although preterm fetuses show chemoreflex-mediated peripheral vasoconstriction in response to asphyxia, it is slower to be initiated compared with near term (51), and indeed, the response to moderate hypoxia is considerably attenuated (19), supporting the hypothesis that there is also a maturational change in baroreflex-mediated sympathetic control of peripheral resistance.

The aim of this study was, therefore, to determine the presence and speed of baroreflex control of RSNA and HR in the fetal sheep at 0.7 of gestation.

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was withdrawn 18 h before surgery. Ewes were given 5 ml of Streptocin [procaine penicillin (250,000 IU/ml) and dihydrostreptomycin (250 mg/ml; Stockguard Labs, Hamilton, New Zealand)] intramuscularly for prophylaxis 30 min before the start of surgery. Anesthesia was induced by intavenous injection of Alfaxan (Alphaxalone, 3 mg/kg; Jurox, Rutherford, New South Wales, Australia), and general anesthesia was maintained using 2–3% isoflurane in O2. The depth of anesthesia, maternal heart rate, and respiration were constantly monitored by trained anesthetic staff. Ewes received a constant infusion isotonic saline drip (at an infusion rate of ~250 ml/h) to maintain fluid balance.

Briefly, fetal hindlimbs and abdomen were exposed through a midline incision, and a small incision in the uterus (35, 36). The left femoral artery and vein were isolated and catheterized with polyvinyl catheters (inner diameter of 1.0 mm and 0.8 mm) to measure mean arterial blood pressure (BP) and mean venous pressure (VP), respectively. The left kidney was exposed via a retroperitoneal incision, the renal sympathetic nerve was visualized with a surgical microscope (OPMI IFC, Zeiss, Oberkochen, Germany), and the electrode coils of a telemetry-based implantable nerve amplifier (Telemetry Research Limited, www.telemetryresearch.com, Auckland, New Zealand) were coiled around the nerve. The electrode and nerve were insulated from the surrounding tissues with a coat of silicone elastomer (Kwik-sil; World Precision Instruments, Sarasota, FL) (4, 9). The implantable amplifier was then secured to the back of the fetus. To ensure that continuous signals were recorded, an aerial was also secured on the back of the fetus (9). The uterus was then closed in layers, and a second incision was made to expose the fetal head and upper chest. Polyvinyl catheters were placed in the fetal right brachial artery, for withdrawal of predural arterial blood samples, right brachial vein, for drug infusion, and the amniotic sac. ECG electrodes (Cooner Wire, Chatsworth, CA) were sewn across the fetal chest to record fetal HR. Polyvinyl catheters (inner diameter of 1.0 mm and 0.8 mm) to measure mean arterial blood pressure (BP) and mean venous pressure (VP), respectively. The fetal laparotomy skin incision was infiltrated with a local analgesic, 10 ml 0.5% bupivacaine plus adrenaline (AstraZeneca, Auckland, New Zealand). All fetal catheters and leads were exteriorized through the maternal flank. The maternal long saphenous vein was catheterized to provide access for postoperative maternal care and euthanasia.

Postoperatively, sheep were housed together in separate metabolic cages with access to water and food ad libitum. They were kept in a temperature-controlled room (16 ± 1°C, humidity 50 ± 10%), in a 12:12-h light-dark cycle. Antibiotics were administered daily to the ewe [600 mg iv of benzylpenicillin sodium (Novartis, Auckland, New Zealand) and 80 mg iv of gentamicin (Pharmacia and Upjohn)]. Fetal catheters were maintained patent by continuous infusion of heparinized saline (20 U/ml at 0.15 ml/h), and the maternal catheter was maintained by daily flushing.

**Experimental design.** Experiments were conducted at 102 ± 1 days gestation, at least 24 h after the end of surgery. Brain development at this age is equivalent to the preterm human fetus (28–32 wk gestation) (29). HR, BP, VP (corrected by subtraction of intra-amniotic pressure), ECG, and RSNA were recorded and saved continuously to disk for off-line analysis using custom data acquisition programs (LabView for Windows; National Instruments, Austin, TX). Data recording was begun 12 h before the start of the experiment.

To test baroreflex control, blood pressure was manipulated using the vasoactive drugs, sodium nitroprusside (SNP; Sigma-Aldrich New Zealand, Auckland, New Zealand) and phenylephrine (PE; Sigma-Aldrich). Thirty minutes before the drug challenges, an arterial blood sample was taken for predrug pH, blood gas, base excess (Ciba-Corning Diagnostics 845 blood gas analyzer and co-oximeter; Cambridge, MA), glucose, and lactate measurements (YSI model 2300; Yellow Springs, OH). All fetuses (n = 7) had normal biochemical variables for this gestation before the baroreflex challenges, according to our laboratory standards (36). Each fetus then received one bolus infusion of PE (50–100 μg) and one bolus infusion of SNP (25–100 μg) in random order. The drugs were made up to a concentration of 250 μg/ml in sterile saline, filtered (Acrodisc syringe filter, Pall, Newquay, Cornwall, UK) and infused intravenously with 3 ml of sterile saline. Before the second drug was infused, blood pressure and heart rate were allowed to return to baseline values (~1 h between each challenge). On completion of the drug infusions, a second blood sample was taken to assess whether there were any biochemical changes associated with baroreflex challenges. At the end of the protocol, the ewe and fetus were killed with an overdose of pentobarbital sodium (9 g iv to the ewe; Pentobarb 300, Chemstock International, Chirstchurch, New Zealand).

**Data analysis.** Off-line physiological data analysis was performed using Labview-based customized programs (National Instruments). Data were averaged in 5-s intervals for the majority of the analysis. Two-second average data were used to assess the detailed changes in HR and BP. RSNA signals were amplified 50,000 times, filtered between 50 and 2,000 Hz, full-wave rectified, and integrated using a low-pass filter with a time constant of 20 ms (4, 9). The analog signals were then digitized and continuously displayed and recorded at 500 Hz. Nerve activity was expressed as a percentage of baseline (baseline was taken as the 15 min before drug infusion), as the absolute level of RSNA is dependent on a number of factors, including the degree of contact of the nerve with the electrodes. Confirmation of the RSNA signal was established by the presence of coordination between the bursting pattern in sympathetic activity and the cardiac cycle on 1-s average recordings of blood pressure and RSNA, obtained using the systolic pressure as a trigger, for 200 epochs (data not shown) (9, 27). Fetuses with excessive movement artefact or ECG interference on the signal were excluded from RSNA analysis (9). The blood pressure signals (Novatrans II, MX860, Medex, Hilliard, OH) were collected at 64 Hz and low-pass filtered at 30 Hz. The fetal ECG was analog filtered between 0.05 and 80 Hz and digitized at 512 Hz.

Overall, changes in BP following each drug infusion were followed by reciprocal changes in HR; however, after a fall in BP, there was a significant delay before HR rose. This systematic shift in the timing of the heart rate response meant that sigmoidal baroreflex curves could not be meaningfully derived; therefore, the responses to SNP and PE were analyzed separately. To compare the timing of the changes in HR and BP between SNP and PE infusions, the time to “half the maximum change” was determined using the individual maxima/minima from 2-s averaged data, and the time difference between 50% change in BP and HR was compared. Control levels for each variable were taken as the average of the 2 min before drug infusion.

**Statistics.** Statistical analysis was performed using SPSS (SPSS, Chicago, IL) and GraphPad Prism (GraphPad Software, San Diego, CA). Changes in BP, HR, and RSNA were tested using repeated-measures ANOVA on 5-s averaged data. Where a significant effect of time was found, post hoc comparisons were made using Dunnett’s multiple comparison test. To evaluate the difference in the timing of the changes in HR and BP between SNP and PE infusions and changes in biochemical variables, data were compared by paired t-test. Statistical significance was accepted when P < 0.05. Data are expressed as means ± SE.
RESULTS

Biochemical measurements before and after baroreflex challenges. Baroreflex challenges were not associated with significant changes in blood gas, pH, base excess, or glucose-lactate status (Table 1).

Changes in fetal BP, HR, and RSNA with SNP and PE infusions. After SNP infusion, BP fell from a baseline of 38 ± 2 mmHg to a nadir of 26 ± 1 mmHg (Fig. 1). The fall was significantly different from baseline 10 s (mean) after the infusion (P < 0.05). Following SNP infusion, there were no significant changes in RSNA (Fig. 1). An example of raw data from one fetus is shown in Fig. 2. Figure 2A shows the coordinated discharges of nerve activity against a quiet background signal under baseline conditions, and Fig. 2B shows RSNA in the same animal at the nadir of BP after SNP infusion. After SNP infusion, HR increased from a baseline of 182 ± 4 to a maximum of 274 ± 8 bpm. This increase was significantly greater than baseline after a mean of 60 s (P < 0.01, Fig. 1) and was slower than the change in BP, with a 31 ± 8 s delay between the 50% changes in BP and HR after SNP infusion [50% rise in BP reached after 31 ± 3 s; 50% HR after 62 ± 9 s (calculated using individual data)].

PE infusion was followed by an increase in BP from a baseline of 38 ± 2 mmHg to a maximum of 58 ± 3 mmHg (Fig. 3). This was significantly different from baseline after 10 s (P < 0.05). There were no significant changes in RSNA. HR fell from a baseline value of 177 ± 4 bpm to a nadir of 116 ± 8 bpm and was significantly different from baseline after 20 s (P < 0.05). The time between the 50% change in BP and HR was significantly shorter in response to PE than after SNP (14 ± 5 s vs. 31 ± 8 s; P < 0.05). For PE, the 50% rise in BP was attained after 25 ± 3 s and 50% HR after 39 ± 4 s.

DISCUSSION

In the adult, baroreflex control of the vasculature via the sympathetic nerves plays a central role in short-term maintenance of blood pressure. Although a sigmoidal relationship between RSNA and BP, similar to that seen in the adult, has previously been reported in near-term fetal sheep (39, 41), the current study found that in the preterm fetal sheep, there were no significant changes in RSNA during blood pressure manipulation, indicating a lack of baroreflex control of peripheral resistance. Further, although the heart rate baroreflex responses were active, the heart rate responses to changes in blood pressure were asymmetrical, with a markedly slower heart rate response to falling BP than to rising BP. Thus, there are significant differences in the baroreflex control of both heart rate and sympathetic nerve activity in the preterm fetus that have important implications for understanding cardiovascular control during gestation.

Traditionally, sympathetic nerve activity has been thought of as having three distinct properties: 1) bursts of activity that are a result of the coordinated firing of many fibers at the same time, 2) coordination of these bursts with the cardiac cycle, and 3) changes in the level of activity in response to changes in blood pressure (10). Work in the 1970s and 1980s showed that entrainment of the bursts of activity with heart rate and the mean changes in sympathetic nerve activity with changes in blood pressure are both baroreceptor-input dependent (3, 45); however, more recently, it has been shown in anesthetized sinoaortic denervated adult rabbits that entrainment of RSNA with the cardiac cycle persists despite loss of baroreflex control of the mean level of RSNA (27), and thus, these phenomena are not necessarily linked. Carotid baroreceptor activity has

Table 1. Fetal arterial pH, blood gases, glucose and lactate values 30 min before the start of baroreflex challenges (baseline) and after the end of the challenges (post baroreflex challenges)

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Post BC</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.38±0.01</td>
<td>7.38±0.00</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>41.6±1.2</td>
<td>42.4±1.2</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>28.2±0.8</td>
<td>28.6±0.9</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.7±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Base excess, mmol/l</td>
<td>–0.6±0.7</td>
<td>–0.6±0.7</td>
</tr>
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</table>

Values are averages ± SE. PaCO₂, fetal arterial partial pressure of CO₂; PaO₂, fetal arterial partial pressure of O₂.
been reported in the preterm fetus (8), and there is evidence that generation centers in the central nervous system are triggered by the baroreceptors to coordinate bursts of RSNA with the cardiac cycle (9); however, the present data suggest that these inputs may not be sufficient to affect mean sympathetic activity. This is broadly consistent with previous direct and indirect data that resting sympathetic tone was low in the preterm fetus (2, 9, 31, 44), suggesting that tonic sympathetic nerve activity is not essential for maintenance of BP early in gestation.

Although RSNA did not increase in response to hypotension, there are preliminary data showing that RSNA in the preterm fetus can substantially increase during severe asphyxia (5). Therefore, in contrast to the term fetus and after birth, in the preterm fetus, neural control of the vasculature via the sympathetic nerves may be activated primarily, if indeed not exclusively, under life-threatening conditions. This is consistent with evidence that the acute decrease in femoral vascular conductance in response to asphyxia is significantly slower in the preterm fetus than near term (51). This may be interpreted as immaturity or insensitivity of a key controlling system; however, it may also be a reflection of the remarkable anaerobic tolerance at this early stage of gestation (19). Supporting this hypothesis, the preterm fetus does not centralize blood flow in response to periods of moderate hypoxia but shows a brisk and coordinated response to a more severe insult, such as asphyxia (as reviewed in Ref. 19).

In contrast to RSNA, the present study provides evidence that HR is under baroreflex control; however, the responses to increasing and decreasing blood pressure were asymmetrical. When blood pressure was increased, there was a brisk fall in heart rate, whereas when pressure was reduced there was a delay before heart rate increased. It is important to appreciate that while asymmetry of the cardiac baroreflex has been reported in the adult (as reviewed in Ref. 52), the delay due to conduction time of the reflex loop is estimated to be just 800 ms in the adult human (33), which is readily accounted for by averaging the heart rate over 1- or 2-s intervals. In contrast, in the preterm fetus, we now report a marked lag between changes in BP and subsequent changes in HR, with a doubling of the time taken for HR to respond between the upward and downward blood pressure changes. Thus, any sigmoidal or linear relationship that we could have “forced” to the data would be potentially misleading compared with the adult baroreflex.

Although the exact mechanisms were not able to be determined in the current study, the delayed heart rate response appears to denote a lack of rapid, fine control of the downward arm of the baroreflex in the preterm fetus. In the adult the increase in heart rate in response to a decrease in blood pressure is rapid and is primarily due to withdrawal of vagal activity (12, 25, 34, 50), although there is some evidence for a more significant role for the sympathetic nervous system (17) or a combination of the two systems (37, 46, 47). In the preterm fetus, the autonomic nervous system is still developing (2, 31, 48), and blockade of resting vagal activity at this gestational age does not significantly alter baseline HR (48). Thus, whereas the quick decrease in HR following phenylephrine infusion most likely is mediated by the well-established vagally mediated response, as previously reported in both the near-term fetus and adult (20, 42, 54), we may reasonably postulate that the delay in HR indicates limited responsiveness of cardiac sympathetic nerve activity (CSNA), unmasked by minimal potential for vagal withdrawal.

Consistent with this, it has previously been shown that the increase in HR in response to mild hypotension in the fetal sheep over the last 35 days of gestation is unchanged by atropine but abolished by propranolol (49). Others have reported that propranolol reduced the maximum HR response to hypotension but did not completely prevent the increase in HR (54), suggesting that both sympathetic and parasympathetic influences are active in near-term fetal sheep. However, resting vagal activity is also higher near term than in the preterm fetus.
maxima/minima from 2-s average data indicate the 50% time of change in HR and BP calculated using the individual and 0.87 gestation in fetal sheep (16). Studies comparing the equine fetuses (32) or no change in sensitivity between 0.76 reported decreasing sensitivity between 0.6 and 0.9 gestation in heart rate, in response to stepwise changes in blood pressure, gestation. Fetal studies that measured the maximum changes in data from fetal sheep on the sensitivity of the baroreflex during that may explain some of the considerable discrepancies in the preterm fetus has immediate methodological implications for systemic circulation. may reflect time required for release of catecholamines and space flight (as reviewed in Ref. 15).

Conversely, intriguingly, we note supportive evidence that reduced hydraulic stress, including extended bed rest (23) and postnatally the baroreflex may be attenuated by conditions of increased age (39). Because these studies contrast to the baroreflex control of RSNA reported in the near-term fetal studies (25, 39, 54). In addition, the present studies were carried out between 24 and 72 h after the end of surgery. The limited recovery time could potentially affect reflex responses; however, previous studies of the baroreflex control in the fetus were either performed acutely (39) or began within 72 h of surgery (21, 42), similar to the present study.

**Perspectives and Significance**

The main finding of this study was that although a cardiac baroreflex was evident, there was no significant baroreflex control of mean RSNA in the preterm fetus. This is in stark contrast to the baroreflex control of RSNA reported in the near-term fetal sheep and after birth (39). Combined with the finding that the heart rate responses to hypotension were markedly slow, these data strongly suggest that baroreflex responses to hypotension are much less effective in the preterm fetus than in the adult. Thus, this supports the hypothesis that attenuation of baroreflex-mediated responses may be a significant contributor to blood pressure instability in preterm neonates, especially in the first few hours after birth (1). It remains to be determined whether this indicates a true immaturity of a key regulatory system or, alternatively, whether this reflects the unique fetal environment. The fetus is hydraulically supported in utero; therefore, we may postulate teleologically that there are not the same hydrostatic demands for rapid adjustment of BP in the preterm fetus as after birth. Although there are no direct data on baroreflex-mediated sympathetic activity in full-term lambs, baroreceptor-mediated changes in heart rate variability increase markedly over the first 10 days of life (53). Conversely, intriguingly, we note supportive evidence that postnatally the baroreflex may be attenuated by conditions of reduced hydraulic stress, including extended bed rest (23) and space flight (as reviewed in Ref. 15).

**GRANTS**

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**Fig. 3.** Renal sympathetic nerve activity (RSNA; expressed as a percentage of baseline), fetal heart rate (HR; in bpm), and mean arterial blood pressure (BP; in mmHg) following an infusion of phenylephrine (PE). Dotted line indicates point of PE infusion. Data are 5-s averages ± SE. The horizontal error bars indicate the 50% time of change in HR and BP calculated using the individual maxima/minima from 2-s average data ± SE. The horizontal bars indicate significant changes from baseline: *P < 0.05, **P < 0.01.

(2, 31, 48). In contrast, at 100 days gestation in fetal sheep, sympathetic innervation of the heart is present but not complete and increases toward term (22). Therefore, speculatively, it is possible that CSNA may not be significantly increased with hypotension at this gestational age, similar to the lack of RSNA response observed in the present study. The increase in heart rate may then be mediated by catecholamine release from the adrenal medulla, and thus, the delay may reflect time required for release of catecholamines and for systemic circulation.

The delay between the decrease in BP and increase in HR in the preterm fetus has immediate methodological implications that may explain some of the considerable discrepancies in the data from fetal sheep on the sensitivity of the baroreflex during gestation. Fetal studies that measured the maximum changes in heart rate, in response to stepwise changes in blood pressure, reported decreasing sensitivity between 0.6 and 0.9 gestation in equine fetuses (32) or no change in sensitivity between 0.76 and 0.87 gestation in fetal sheep (16). Studies comparing the cardiac baroreflex in the term fetus compared with after birth using ramp changes in blood pressure showed a trend toward lower sensitivity with increased age (39). Because these studies calculated maximum heart rate responses to stabilized blood pressure (i.e., asynchronous measurements), they may have missed the initial delay in heart rate responses. Conversely, studies investigating the immediate (synchronous) effects of blood pressure changes on heart rate have shown decreased baroreflex sensitivity in the preterm fetus compared with closer to term (42) or in the fetus compared with after birth (13).

Some potential limitations of the current study should be considered. When comparing between the upward and downward limbs of the baroreflex, it must be appreciated that vasoactive drugs can have direct extravascular effects. In the adult, nitric oxide donors, such as sodium nitroprusside, have previously been suggested to have inhibitory effects on baroreceptor activity affecting the baroreflex response (as reviewed in Ref. 11). However, appropriate baroreflex responses have been extensively documented with these agents in adult and near-term fetal studies (25, 39, 54). In addition, the present studies were carried out between 24 and 72 h after the end of surgery. The limited recovery time could potentially affect reflex responses; however, previous studies of the baroreflex control in the fetus were either performed acutely (39) or began within 72 h of surgery (21, 42), similar to the present study.
REFERENCES


