Pressure pulses and flow velocities in central veins of the anesthetized sheep fetus

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Submitted 8 November 2002; accepted in final form 20 December 2002

Schröder, Hobe J., Mikhail Tchirikov, and Christian Rybakowski. Pressure pulses and flow velocities in central veins of the anesthetized sheep fetus. Am J Physiol Heart Circ Physiol 284: H1205–H1211, 2003. First published December 27, 2002; 10.1152/ajpheart.00969.2002.—The pressure drop and pressure pulses in the isthmus of the ductus venosus (DV) in fetal sheep have not been measured directly and related to flow. In eight acutely anesthetized fetal sheep, a 3-Fr tip pressure transducer (TP) was inserted from the external jugular into the umbilical vein (UV). Ultrasound Doppler flow velocities, TP position, and intravenous pressures were recorded in the UV, DV, and inferior vena cava (VC) while the TP was withdrawn. Flow was steady in the UV, but small pressure fluctuations (<0.4 mmHg) could be detected. Time-averaged pressure dropped 1.9 mmHg (mean; 0.5–3.3 mmHg 95% confidence interval) across the DV isthmus. Pressure pulses increased from 1.7 mmHg (mean; 1.2–2.1 mmHg 95% confidence interval) in the DV to 3.9 mmHg (mean; 1.8–6.0 mmHg 95% confidence interval) in the inferior VC. The pressure wave from the heart arrived later (0.053 s (mean; 0.025–0.080 s 95% confidence interval)) in the isthmus of the DV than in the diaphragmatic inferior VC, indicating a wave velocity of ~1.1 m/s. At all locations, pressures and flow velocities were inversely related.

fetal sheep; pressure wave; flow velocity; ductus venosus

IN THE FETUS, flow is pulsatile in both the venae cavae (VC) (22) and ductus venosus (DV) (10) and normally nonpulsatile in the portal sinus or umbilical vein (UV). Variations of the flow rates and/or velocity patterns predominantly in the DV, and their significance for the well-being of human fetuses, have been investigated by Doppler ultrasound and shown to be of diagnostic value (2–4, 8–10, 25). Cardiac and circulatory dysfunction as disclosed by an abnormal flow pattern may be associated with pressure variations, which at present are mostly unknown. However, only the knowledge of both flow and pressure permit sufficient understanding of physiological and pathological conditions in the central venous circulation (11, 14, 20, 22). Mathematical (1, 6, 7, 18–20) and in vitro models (5, 13) have been used to gain insight into the complex situation, but empirical in vivo data on these pressure-wave forms

METHODS

The experiments with eight pregnant sheep followed the guidelines for animal research of our institution. The animals (median gestational age, 121.5 days) were sedated with xylazine (Rompun; 0.25 mg/kg im) and intubated after intravenous injection of thiopental sodium (Trapanal; 1 g). Inhalation anesthesia was maintained by artificial ventilation with 1% halothane in an O2-N2O mixture.

The animal was placed on a heating pad (38°C) in the supine position, and the maternal abdomen was opened by a midline incision. The fetal head was delivered after hysteromy, and the myometrium was attached loosely to the fetal neck to avoid losses of amniotic fluid. From the right external jugular vein, a catheter was inserted into the UV as described previously (27). The catheter allowed introduction of a tip pressure transducer with side openings. In three animals 3-Fr Sensodyne pressure transducers (Braun) and in five animals 3-Fr Millar pressure transducers (model SPR-524) were used (both ~3 db, >5 kHz). The right carotid artery was catheterized for pressure (Ohmeda P23XL) and

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heart rate measurements and for fetal blood gas analysis (ABL 710, Radiometer Copenhagen). A fluid-filled catheter connected to a pressure transducer (Ohmeda P23XL) was inserted into the amniotic cavity to monitor amniotic fluid pressure.

In the first three experiments, only the ECG could be recorded in conjunction with the Doppler signal, and ECG electrodes were attached to the fetal scalp and myometrium to allow synchronization of pressure and Doppler velocity signals. In later experiments, the signal of the tip pressure transducer was displayed directly on the screen of the ultrasound system (alternating current-coupled "physiological" input, Acuson Aspen). In one animal, after insertion of the first tip pressure transducer (Millar), a second Millar catheter was introduced as described above in an attempt to measure intravascular pressure differences.

**Experimental Procedures**

Central venous, arterial, and amniotic pressures and the ECG signals were continuously acquired by a MP 100 data-acquisition system (sampling rate 250 Hz, in two experiments 1,000 Hz; Biopac Systems) and stored on disk. Either the ECG or the signal from the tip pressure transducer amplifier was led into the ultrasound system and displayed on screen simultaneously with the Doppler velocity signal. All output to the screen was continuously recorded on videotape.

The ultrasound probe was placed directly on the uterine wall, and the tip of the pressure transducer was localized. The Doppler gate was positioned to cover the vessel as close to the pressure transducer tip as possible (within 0.5 cm) but avoiding direct inclusion. Velocity pulses were recorded when the signal quality was judged as sufficient. The parameters of the maximum velocity envelope curve (minimum \( V_{\text{min}} \), maximum \( V_{\text{peak}} \), and time-averaged mean velocities (TAMX)) were determined. The tip pressure transducer was then withdrawn, and its new position was verified by ultrasound B-scan. Venous pressures and flow velocities were observed at the following five positions, which covered a distance of 6–8 cm: the UV, 1 cm upstream of the isthmus; DV1, immediately downstream of the isthmus; DV2, outlet; VC1, the inferior VC close to the outlet of the DV; and VC2, the inferior VC close to the right atrium.

The experiments were terminated by intravenous injection of 15 ml of euthanasia solution T61 (Hoechst) into the ewe. The fetus was removed, and the tip pressure transducer was calibrated.

**Calibration of Tip Pressure Transducers**

The tip pressure transducers were calibrated before and immediately after an experiment. Typically, there was a baseline shift ranging from 2 to 6 mmHg but a constant "gain" (slope of the calibration line). Therefore, pressure changes that occurred within 1 min or less were measured accurately.

The arterial and amniotic pressure recording systems were also calibrated with zero adjustments during the experiment.

**Data Processing**

**Pressures.** Digitized data were evaluated using Acqknowledge analysis software (Biopac Systems). On the basis of postexperiment calibration data, all pressure signal voltages were transformed to pressure values (in mmHg). Depending on the signal-to-noise ratio, pulsatile venous pressure signals mostly from the UV were digitally filtered by a 15- or 25-Hz low-pass filter, which did not affect phases. Pressure pulses were measured during time intervals when no ultrasonographic recording took place. The pressure pulse (difference between minimum and maximum pressure during one cardiac cycle) of venous pressure was determined over five cardiac cycles to minimize the influence of ventilation (cf. Fig. 1).

Corresponding characteristic points (peak or trough values) of the venous pressure pulses at positions DV1 to VC2 were identified. The time difference between the corresponding point and the beginning of the carotid pressure rise (time of end-diastolic pressure as reference) was then measured at positions DV1 to VC2 (Fig. 3). When two Millar catheters were inserted, corresponding points at two different locations could be observed simultaneously (Fig. 4). When the ECG signal was available (3 animals), the time difference between the R-peak and the end-diastolic arterial pressure rise was determined to obtain information on the variability of the timing of the arterial pressure rise.

**Doppler flow velocities.** On the basis of the maximum velocity envelope curve, the \( V_{\text{min}} \), \( V_{\text{peak}} \), and TAMX of one to three consecutive cardiac cycles (depending on the signal quality) were determined (cf. Figs. 5 and 6). From these points, the pulsatility index \( [PI] = (V_{\text{peak}} - V_{\text{min}})/\text{TAMX} \) was calculated. Doppler angles averaged 46° (mean; 42–50° 95% confidence interval) and did not exceed 60°.

**Motion artifacts.** The tip of a Millar catheter was inserted 5 cm into water and oscillated perpendicular to the sensitive area (1–3 cm/s). Apparent pressure decreases and increases of maximal 0.5 mmHg were observed. The time-averaged mean pressure was accurately recorded as 5.0 cm H2O.

**Statistics**

Average results are described as means and 95% confidence intervals (in parentheses) unless stated otherwise. To test for differences between the five locations, ANOVA and Fisher’s post hoc test were performed (Statistica, Statsoft; Tulsa, OK). \( P < 0.05 \) was accepted as significant.

**RESULTS**

Gestational age of the fetuses ranged from 112 to 127 days (term 145 days), and mean body weight was 2.5 kg (1.6–3.3 kg). In four twin pregnancies, one of the two siblings was included. The experiments lasted between 45 and 150 min.

Fetal arterial blood gas values approximately in the middle of the experimental procedure were \( pH 7.23 \) (7.16–7.30 pH), \( PO_2 \) 25.3 mmHg (19.6–31.0 mmHg), \( PCO_2 \) 66.2 mmHg (55.1–77.2 mmHg), and base excess −1.1 mmol/l (−3.4 to 1.2 mmol/l), which indicates a moderate, mostly respiratory acidosis.

A typical pressure recording is displayed in Fig. 1 when the tip pressure transducer (middle) moved (as judged from the ultrasound B-scan) from the portal sinus into the DV with a sudden small pressure drop (arrow) at the isthmic region. At this moment and position, pressure pulses appeared, which were linked to the arterial pressure pulses (Fig. 2, see Figs. 5 and 6). In five experiments with these three criteria, the time-averaged pressure drop across the isthmus of the DV was 1.9 mmHg (0.5–3.3 mmHg). This pressure drop was significant (paired \( t \)-test). In three experiments, the above signs could not be detected in combination.

_AJP-Heart Circ Physiol_ • VOL 284 • APRIL 2003 • www.ajpheart.org
In the UV, small pressure pulses were visible in four experiments (Fig. 2, left); they could, however, not be detected in two animals and were questionable in another two animals. The occurrence of detectable pressure pulses was not related to the fetal arterial PO2. Flow measured by ultrasound Doppler was nonpulsatile in the UV with one exception, where pressure pulses were also observed.

Mean pressure pulses in the UV, DV1, DV2, VC1, and VC2 are summarized in Table 1; the pressure pulse at VC2 was significantly larger than that at DV1.

Fig. 1. Pressures in the carotid artery (top trace), umbilical vein (UV; left of arrow) or ductus venosus (DV; right of arrow, middle trace), and amniotic fluid (bottom trace). The arrow shows where the tip of the tip pressure transducer had suddenly moved from the portal sinus (UV) into the DV. Associated with the pressure drop was the occurrence of distinct and stable pressure fluctuations, with a time course as shown in Fig. 2, right. Occasionally and with intervention, small pressure pulses were visible in the UV also (see text and Fig. 2, left). Note the influence of artificial maternal respiration on all recording traces.

 Corresponding points of the venous pressure curve were observed 53 ms (25–80 ms) later at DV1 than at VC2 (cf. Fig. 3). Appearance at DV2 was 37 ms (21–52 ms) and at VC1 was 23 ms (9–37 ms) later than that at VC2. Nonparametric γ-statistics (Statistica) showed that it was significantly more probable that the tip location and time differences were in the same order than not.

Table 1. Pressure pulses in fetal central veins

<table>
<thead>
<tr>
<th></th>
<th>Mean Values</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>0–0.4</td>
<td></td>
</tr>
<tr>
<td>DV1</td>
<td>1.7</td>
<td>1.2–2.1</td>
</tr>
<tr>
<td>DV2</td>
<td>1.8</td>
<td>1.6–2.1</td>
</tr>
<tr>
<td>VC1</td>
<td>2.3</td>
<td>1.3–3.3</td>
</tr>
<tr>
<td>VC2</td>
<td>3.9</td>
<td>1.8–6.0</td>
</tr>
</tbody>
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Shown are the pressure pulses (peak to peak amplitudes, in mmHg) in the umbilical vein (UV), ductus venosus (DV), and inferior vena cava (VC). The pressure pulse at VC2 was significantly higher than that at DV1. The amplitude of pressure fluctuations in the UV is an estimate only; see text for details. CI, confidence interval.

Fig. 2. Venous pressure pulses (bottom traces) in the UV (left) and DV immediately downstream of the isthmus (right); same recording as in Fig. 1. Venous pressure recordings were filtered. In this case, pressure pulse in the UV was ~20% of the pulse in the DV. It appears that in the UV, pressure pulses were related to arterial pressures (top traces), but note the difficulty in identifying corresponding points on the pressure curves.

Fig. 3. Synopsis of pressures and ECG (peak of the R wave) during one cardiac cycle. R wave peaks were used to synchronize intravascular pressures in the DV, vena cava (VC), and carotid artery. The arrows indicate corresponding points of the venous pressure waves that were related to the a wave of the velocity profile (atrial contraction, cf. Fig. 6). Note that other peaks or troughs of the DV1 trace were delayed in time as well with regard to the VC2 trace.
mmHg (51.5–55.9 mmHg), and the apparent mean amniotic fluid pressure was 11.1 mmHg (9.2–12.9 mmHg).

In three experiments, the time intervals between the R wave peak and the increase of the systolic arterial pressure (cf. Fig. 3) were 51 ms (45–56 ms), 62 ms (59–65 ms), and 54 ms (52–57 ms). There were no consistent changes related to the position of the tip pressure transducer.

**DISCUSSION**

**Pulsatile Venous Pressure**

The present study confirms and expands the results of two previous reports (21, 22) on fetal sheep (chronic preparation). A mainly inverse relationship between the biphasic time course of pressure and flow was observed in the superior VC (22). From its temporal relation to arterial pressure and the ECG R wave, the large and short-lasting peak of venous pressure (cf. Fig. 5) was thought most likely to be caused by atrial contraction and to cause the “a-depression” in the flow profile. It is apparent from the figures (22) that pressure pulses normally were in the range of 10–15 mmHg and that flow pulsatility matched pressure pulsatility.

The present results confirm the basic flow and pressure profiles in the inferior VC and their inverse relationship (Fig. 5). Mean pressure pulses were distinctly lower (~4 mmHg, Table 1) than reported previously, however (22). This may reflect the differences between a chronic and an acute preparation or the heavily invasive instrumentation with flow probes, which may impede venous return.

Our data demonstrate, moreover, that in the DV, pressure is also pulsatile with a lesser pressure pulse than in the inferior VC (Table 1). Again, pressure and flow pulsations are inversely related (Fig. 6). The differences in pressure pulses between the DV and inferior VC (Table 1) are reflected as differences in flow pulsatility (Table 2), which have also been observed by others (24).

There are no other direct measurements of DV pressure pulses available. On the basis of physical principles, model calculations predict pressure pulses of 1.3 mmHg (approximately from 2.8 mmHg minimum to 4.1 mmHg peak pressure; UV pressure constant at 4.5 mmHg) (1, 19). These model estimates are close to our measurement of 1.7 mmHg (Table 1), which may improve the confidence in the results of this mathematical model.

It cannot be excluded that movement artifacts affect the pressure pulses to some extent. The predicted effect, however, is moderate (~0.5 mmHg) even with wide tip oscillations (1–3 cm). Tip movements (2–4 Hz) typically did not exceed 0.3 cm, as judged from the video recordings.

Because pressure in the UV (driving pressure) was mainly constant, flow variations in the DV and in the inferior VC were caused by changes in the opposing pressure, which explains the inverse relationship between pressure and flow. Pressure profiles distant from the heart were delayed with respect to more downstream locations, which is in agreement with the delay of the flow pulses (21). On average, the pressure wave arrived 53 ms later at the entrance of the DV (DV1) than at the inferior VC close to the heart (VC2), and the average time delay for the positions in between varied accordingly. Simultaneous pressure measurements at different locations in one experiment are in agreement. In Fig. 4, top, catheter MIL1 was located at DV1 and MIL2 was located in the inferior VC (in between VC1 and VC2). The time difference between corresponding points (arrows) was ~30 ms (dotted vertical line). About 50 min later (Fig. 4, bottom), both catheter tips were at the same location in the VC, and the corresponding points now occurred at the same time.

On the basis of ultrasound B-scans and postmortem observations, the average distance from VC2 to DV1 was taken as 6 cm. The mean velocity of the pressure wave was then calculated to be 1.1 m/s. This agrees...
well with the pulse wave velocity of 1–3 m/s, which was derived from the measurement of vascular stiffness of the DV and UV in an in vitro model (5) (fetal sheep). In adult animals, values of 1.2–2.5 m/s in the thoracic VC have been reported (15). Nonparametric γ-statistics (Statistica) confirmed the relation between time delay and location, but data scatter prevented establishment of statistically significant differences between time delays using ANOVA. There are several reasons that explain the large variation in the measurement of time differences. Time resolution was restricted by the sampling rate (250 Hz or 4 ms), but most influential were variations in the pressure profile with shifts in the position of the respective peaks or nadirs (cf. Fig. 3). It is possible that motion artifacts of the tip pressure transducers contributed to these displacements. As judged from the variation of the time difference between the R peak and the end-diastolic pressure rise (10% or less of the mean value), the time delay measurements were not biased by systematic shifts of the time reference point (end-diastolic carotid pressure). Because time differences

Fig. 5. Example of an ultrasonographic Doppler velocity recording (step line) in the inferior VC close to the fetal heart. Cavai pressure (top trace) is also shown (peak-to-peak value 3.3 mmHg), and carotid artery pressure is overlaid to allow the assessment of the ventricular cycle (bottom trace). Vertical lines indicate the position of a wave of flow velocity, which is generated by atrial contraction. Note the small time lag between the venous pressure peak and a wave nadir, and the small venous pressure peak, which has no detectable flow counterpart. The box displays the results of Doppler velocity evaluation. Max, maximum; min, minimum; TAMX, time-averaged mean velocity; PI, pulsatility index; AT, acceleration time.

Fig. 6. Recording of Doppler velocity (step line) and intravascular pressure (top trace; peak-to-peak pressure amplitude is 1.1 mmHg) close to the outlet of the DV. Vertical lines mark the position of a wave in the velocity profile; they do not coincide exactly with the diastolic venous pressure peaks, which are generated by atrial contractions. Pressure in the carotid artery is overlaid (bottom trace). Note that the venous pressure is basically a mirror image of the maximum velocity curve. The box shows Doppler evaluation.
were determined, the constant delay between the true beginning of the right ventricular systolic ejection phase and the systolic (carotid artery) pressure rise in the external pressure transducer is of no concern.

Pressure pulses existed occasionally in the UV (Fig. 2, left). Their amplitude was small (–0.2–0.4 mmHg in 4 animals), but the pulses were related to the arterial pressure pulses and were not random. These pressure fluctuations are likely transmitted from the DV, and it has been shown in model calculations (7) that the pulse amplitude depends on the compliance of the UV and on the diameter of the isthmus. Both are increased in fetal hypoxemia, as are UV flow pulsations (9), but our data did not reveal an influence of the arterial PO2 on the occurrence of UV pressure pulses. Pressure pulsations may be imposed by the umbilical arteries on the UVs and then travel from the cord toward the portal sinus. It is also possible that motion artifacts were generated, although the catheter tips appeared to move very little in the UV.

**Time-Averaged Venous Pressure**

When the site of venous pressure recording was shifted from the portal sinus into the DV (DV1), there was in most cases a sudden pressure drop, which amounted to ~2 mmHg (Fig. 1, arrow). This value is the first direct measurement of the isthmic pressure gradient and puts previous indirect estimates in human fetuses [0.3–1.3 mmHg (28) and 0.5–2.5 mmHg (12)] and model calculations (6, 19) with comparable results on a firm basis. In fetal sheep, a pressure drop of 3.1 ± 0.5 mmHg from the portal sinus to the diaphragmatic inferior VC was determined, which includes the isthmic region (17).

In three fetuses, the three criteria to identify the pressure drop across the isthmus could not be met. In one case, the pressure transducer tip was pushed into a small branch of the UV during ultrasonographic measurements. The tip shifted directly into the DV when the catheter was withdrawn with an increase of time-averaged pressure instead of a decrease. In another case, time-averaged pressure dropped 1.3 mmHg, but pressure pulses did not appear or increase immediately. In the third fetus, the tip pressure transducer could not be visualized during its passage from the portal sinus into the DV.

Because of baseline shifts of the tip pressure transducer and the peculiar experimental situation (fetal head exteriorized, uterus incompletely closed) that prevents the accurate measurement of the reference (amniotic) pressure, we are unable to present reliable data on time-averaged continuous central venous, arterial, and amniotic fluid pressures.

**Relationship Between Pressure and Flow Pulses**

As demonstrated in Figs. 5 and 6, a transient increase of local pressure was associated with a transient decrease of flow velocity and vice versa, although minor differences between both profiles were sometimes visible. Typically, the pressure a wave before onset of the ventricular systole was followed by a smaller systolic pressure rise of longer duration (Figs. 5 and 6), as described in a previous report (22). However, variations of this basic pattern did occur, and, especially in the DV close to the isthmic region (DV1), both pressure peaks could be of similar size and duration.

Small time differences (up to about 50 ms; cf. Fig. 5 and 6) between corresponding characteristic points of the flow and pressure wave could also be detected, but we could not find systematic phase differences. These time differences were seen, although our technique allows us to observe pressure and flow velocity simultaneously and at nearly identical locations, which is not possible with outside pressure transducers (22). Model calculations (19) also appear to predict small time differences between peaks and troughs of the flow and pressure wave. The phase shifts (cf. Fig. 6) indicate that, as in the arterial system, pulsatile flow in fetal central veins is affected by viscous, inertial, and elastic forces. Although, in principle, the simultaneous observation of pressure and velocity pulses would permit a dynamic analysis of the fetal central venous circulation (e.g., calculation of vascular impedances; cf. Ref. 7), the technical requirements to do so are not sufficiently satisfied at present.

In conclusion, contractions of the right atrium and ventricular movements (22) generate a pressure wave that travels at a speed of 1.1 m/s to the DV. In the UV upstream of the isthmus, the size of the pressure pulse suddenly decreases (from 1.7 mmHg in the DV to 0–0.4 mmHg), whereas the time-averaged pressure increases by ~2 mmHg. When pulsatile flow and pressure are observed at the same location, local pressure rises are associated with flow reductions and vice versa. Differences in pulsatility of pressure are reflected as differences in the pulsatility of flow. In some aspects, the
geometry of central veins in the ovine fetus (23) is different from the human situation where, for example, the DV outlet almost directly connects with the right atrium (9). Because flow velocity and velocity pattern in the sheep fetus (26) are very similar to velocities in the DV of human fetuses, it seems likely that our results are valid in principle also for the human fetus.

The instrumentation of the sheep fetus with thin (3-Fr), moveable tip pressure transducer catheters and external ultrasonographic Doppler flow measurements is less invasive than previously reported (22). It can be used also in chronic fetal sheep experiments (21). This may help to explore, in nonanesthetized fetuses, the effects of experimental cardiac failure, for example, on flow and pressures in fetal central veins, and thus to improve for diagnostic purposes the understanding of pathological flow-velocity patterns.

We are grateful to Acuson (Nuremberg, Germany) and Advanced Technology Laboratories (Munich, Germany), who made the ultrasonographic equipment available. The editorial help of G. Power (Perinatal Biology, Loma Linda, CA) is gratefully acknowledged.

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