Calcitonin gene-related peptide increases pulmonary blood flow in fetal sheep

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De Vroomen, Maartje, Yasushi Takahashi, Christine Roman, and Michael A. Heymann. Calcitonin gene-related peptide (CGRP) may play a role in regulation of pulmonary vascular tone in adults. We set out to establish whether or not CGRP has any effect on the fetal pulmonary circulation. Hemodynamic effects of exogenous CGRP were studied in seven near-term fetal sheep. Single CGRP injections into left pulmonary artery (LPA), compared with acetylcholine, and five repeated CGRP injections were studied. Single CGRP injections (1.36 ± 0.13 µg/kg) increased LPA blood flow (transit time ultrasound) significantly, from 26 ± 22 to 202 ± 86 ml/min (P < 0.05), and decreased pulmonary and aortic pressures, from 58 ± 5 to 48 ± 6 mmHg and from 56 ± 3 to 46 ± 5 mmHg, respectively (P < 0.05). LPA resistance decreased from 3.69 to 0.24 mmHg·min·ml⁻¹ (P < 0.05). These changes were similar to those with acetylcholine. Five CGRP injections at 5-min intervals increased LPA flow significantly, in stepwise fashion, and LPA resistance decreased. Heart rate increased stepwise, without changes in pulmonary or carotid arterial pressures. Exogenous CGRP is a potent pulmonary vasodilator in fetal sheep and increases pulmonary flow. CGRP-induced increases in heart rate are not secondary to decreased systemic blood pressure but reflect a positive chronotropic effect. These findings suggest a role for endogenous CGRP in the remarkable decrease in pulmonary vascular resistance during the transition to extrauterine life.

IN THE FETUS, gas exchange occurs not in the lungs, but in the placenta. Consequently, fetal pulmonary blood flow is low. At the time of birth, with the onset of pulmonary ventilation, pulmonary blood flow increases rapidly by ~10-fold (18, 31). However, the mechanisms involved in this dramatic change still are the subject of controversy. Previous animal studies suggest that a number of factors regulate the normal increase in pulmonary blood flow at birth, including mechanical factors and the release of various vasoactive substances. Mechanical factors, such as lung distension, probably increase pulmonary blood flow through the cyclooxygenase pathway by stimulating prostacyclin production (6, 21, 34). Vasoactive substances, such as ATP, acetylcholine, or bradykinin, probably increase blood flow through receptor-mediated stimulation of nitric oxide production (2, 7, 15, 17, 20, 33).

Calcitonin gene-related peptide (CGRP), a gene product of alternate splicing of calcitonin mRNA (3, 25), is widely distributed in the nervous and cardiovascular systems of humans and other species. Previous studies have shown that this peptide has a diverse range of pharmacological activity, that it is a potent vasodilator (5, 23), and that exogenously administered CGRP induces a profound vascular response in humans and other species (4, 9, 12, 13). Because CGRP receptors were identified in high concentrations in the intima and media of lung vessels (24), this peptide may play an important role in regulating pulmonary blood flow (19, 24, 26). A recent study found substantial concentrations of CGRP in both umbilical cord blood and in blood from neonates (28); however, the possible role of CGRP in regulating pulmonary blood flow in the perinatal period has not been examined. The goal of this study thus was to determine whether or not exogenously administered CGRP has any effect on pulmonary blood flow in the chronically instrumented fetal sheep.

METHODS

Animals. We studied seven mixed Western fetal sheep at 125–129 days gestational age (full term is ~145 days). Animal care and the study design followed the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (Department of Health and Human Services Publication No. (NIH) 85–23, Revised 1985) and were approved by the Committee on Animal Research of the University of California, San Francisco.

Surgical preparation. The surgical preparation was performed as described previously (17, 31, 34). Briefly, after administering ketamine anesthesia to the ewe, we performed a midline laparotomy and exposed the fetus through a small uterine incision. After administering local lidocaine anesthesia to the fetus, we made a skin incision in the fetal forelimb and advanced catheters into the ascending aorta from the brachial artery and into the superior vena cava from the brachial vein. We closed the skin incision in the forelimb and then made a new incision over the fetal left chest. We performed a left thoracotomy and advanced a catheter into the ascending aorta from the internal mammary artery. After opening the pericardium, we inserted catheters directly into the main and left pulmonary arteries and into the left atrium. We infiltrated Formalin (10%), colored with a few drops of sterile methylene blue solution, into the adventitia and media of the ductus arteriosus to prevent vasoactivity during the subsequent experimental protocol (29). For continuous measurement of left pulmonary arterial blood flow, we placed a 4- to 6-mm ultrasonic transit time Doppler flow transducer around the left pulmonary artery. After dosing the thoracotomy and fetal skin incision, we replaced amniotic fluid losses with warm saline and instilled antibiotics. We then placed a catheter in the amniotic cavity and closed the uterine incision. All vascular catheters were filled with heparin sodium, sealed, and exteriorized with the transducer cable to the ewe’s left flank. We closed the laparotomy in layers and returned the ewe to the cage for recovery. Antibiotics were
administered daily into the amniotic cavity and intravenously to the ewe.

Experimental protocol. We performed experiments 1–2 days after surgery. The ewe was placed in a study cage and allowed free access to alfalfa pellets and water during experiments. After a steady-state period of at least 20 min, we injected 2 μg acetylcholine (Miochol, Iolab Pharmaceuticals, Clairmont, CA) as a bolus (over ~5 s) into the left pulmonary artery to confirm normal vasoactivity of the pulmonary vascular bed mediated through endothelial nitric oxide production (7, 33). The acetylcholine-induced increase in fetal pulmonary blood flow has been well characterized (7, 33), and thus we used the responses to acetylcholine as the standard against which to assess the subsequent effects of CGRP. After pulmonary arterial blood flow and blood pressure had returned to baseline value, we injected 5 μg CGRP (Sigma Chemical, St. Louis, MO) as a bolus (over ~5 s) into the left pulmonary artery. We then repeated this CGRP injection every 5 min for a total of five injections.

We measured left pulmonary arterial blood flow, phase and mean systemic (ascending aortic) and pulmonary arterial blood pressure, mean left atrial pressure, and heart rate throughout the experiment. Left pulmonary arterial blood flow was measured with an ultrasonic flow transducer and flow meter (Transonic Systems, Ithaca, NY). Systemic and pulmonary arterial blood pressures and left atrial pressure were measured with Statham P23 Db strain gauge transducers (Statham Instruments, Oxnard, CA). Heart rate was measured with a cardiotachometer triggered from the phasic ascending aortic pressure tracing. All measurements were recorded continuously on a direct-writing polygraph (Gould, Cleveland, OH). In addition, we obtained blood samples before and after the experiment from the ascending aorta to determine pH, PCO2, and PO2 (Corning Medical, Medfield, MA), hemoglobin concentration, and a hemoglobin O2-binding capacity of 1.35 ml/g. Differences in these variables before and after the experiment were analyzed by the paired sample t-test. We considered statistical significance present when P < 0.05. Data are presented as means ± SD, except for those presented in a box plot.

RESULTS

Fetal body weight ranged from 3,250 to 4,150 g (median, 3,650 g). Fetal ascending aortic blood gases before and after the experiments are summarized in Table 1. There was a significant decrease in pH and increase in Pco2 and lactate after the experiments. Hemoglobin decreased ~12% after the experiments, but this was not statistically significant.

The changes in left pulmonary arterial blood flow with the first injection of CGRP, compared with the changes with acetylcholine, are shown in Fig. 1. The concomitant changes in other variables are summarized in Table 2. In all seven fetal sheep, exogenously administered CGRP significantly increased fetal pulmonary arterial blood flow, which returned toward and was not significantly different from baseline after 5 min. Mean pulmonary arterial and ascending aortic pressures as well as calculated left pulmonary arterial resistance fell significantly after CGRP administration, and although they were consistently slightly lower, were not significantly different from baseline by 5 min. There were no significant changes in heart rate or in left atrial pressure obtained in five of the seven fetuses. After acetylcholine injection, heart rate decreased significantly, but the other hemodynamic changes were similar to those observed with CGRP.

With the five repeated bolus injections of CGRP, we found a stepwise increase in left pulmonary arterial blood flow (Fig. 2). The results of the regression analyses are shown in Fig. 3. Over the 25-min study period, there was a significant linear increase in left pulmonary arterial blood flow (P < 0.01, r = 0.48, y = 8.8x +

### Table 1. Fetal ascending aortic blood gas analyses before and after the experiments

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<th></th>
<th>pH</th>
<th>Pco2, kPa</th>
<th>PO2, kPa</th>
<th>Hb, g/dl</th>
<th>O2 Saturation, %</th>
<th>O2 Content, ml/dl</th>
<th>Lactate, mM</th>
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<tr>
<td><strong>Before</strong></td>
<td>7.37 ± 0.03 (7.33–7.41)</td>
<td>56.2 ± 3.5 (52.7–63.4)</td>
<td>18.2 ± 2.2 (14.5–21.1)</td>
<td>11.1 ± 2.1 (7.7–12.8)</td>
<td>51.1 ± 12.6 (31.2–64.7)</td>
<td>7.8 ± 2.8 (3.8–11.2)</td>
<td>3.28 ± 2.69 (1.49–8.62)</td>
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<tr>
<td><strong>After</strong></td>
<td>7.30 ± 0.03* (7.24–7.33)</td>
<td>62.7 ± 5.5* (56.6–70.6)</td>
<td>18.9 ± 3.5 (15.2–24.8)</td>
<td>9.8 ± 2.1 (6.6–12.5)</td>
<td>51.7 ± 10.6 (35.1–66.5)</td>
<td>6.8 ± 1.7 (3.6–8.6)</td>
<td>4.89 ± 3.03* (2.54–10.91)</td>
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Values are means ± SD, with minimum to maximum range given in parentheses. Hb, hemoglobin. *P < 0.05 comparing measurements before experiments with those obtained after experiments.
CGRP decreases pulmonary vascular resistance significantly in the sheep fetus, exogenously administered (19, 24, 26). The present study now also demonstrates the potent pulmonary vasodilator properties of this peptide in adult animals have been reported previously. CGRP receptors in the intima and media of lung vessels (24) and the potent pulmonary vasodilator properties of this peptide in adult animals have been reported previously (19, 24, 26). The present study now also demonstrates that in the sheep fetus, exogenously administered CGRP decreases pulmonary vascular resistance significantly, thus increasing pulmonary blood flow.

DISCUSSION

CGRP is among the most potent of vasodilating substances; femtomolar amounts of this peptide injected into animals induce a profound vascular response (4, 9, 12, 13, 23). The presence of CGRP receptors in the intima and media of lung vessels (24) and the potent pulmonary vasodilator properties of this peptide in adult animals have been reported previously (19, 24, 26). The present study now also demonstrates that in the sheep fetus, exogenously administered CGRP decreases pulmonary vascular resistance significantly, thus increasing pulmonary blood flow.

To explain the increase in fetal pulmonary blood flow after CGRP injection, three main factors should be considered: pulmonary vascular resistance, heart rate, and right ventricular stroke volume. Change in resistance in the ductus arteriosus is another important factor in the fetal circulation but was negated in this study by removal of any reactivity due to the effect of Formalin infiltration around the ductus arteriosus (29). In all seven fetal sheep, pulmonary arterial blood pressure and calculated vascular resistance decreased significantly after CGRP injection (Table 2). Therefore, CGRP-induced pulmonary vasodilation and the concomitant decrease in pulmonary vascular resistance are likely the main cause of the increase in fetal pulmonary arterial blood flow. Increases in heart rate and right ventricular stroke volume probably had minimal effect on pulmonary blood flow. First, although pulmonary blood flow increased nearly eightfold, heart rate increased <10%, without statistical significance. Second, although the significant decrease in systemic arterial pressure might indicate a decrease in afterload (Table 2), fetal ability to increase stroke volume is known to be limited (11, 32). Because some studies suggest an inotropic cardiac effect of CGRP (13, 14), further study is necessary to evaluate how CGRP affects the fetal and premature heart.

The vasodilatory action of acetylcholine in both systemic and pulmonary vessels is well known (33). Likewise, in this study, acetylcholine infusion into the left pulmonary artery significantly decreased both pulmonary and systemic arterial blood pressure by 19.0 and 18.5%, respectively. Hemodynamic changes after CGRP injection were quite similar to those after acetylcholine (Table 2), and pulmonary and systemic arterial blood pressure decreased almost equally after the first CGRP injection, 17.2 and 17.9%, respectively, even though CGRP was injected selectively into the left pulmonary artery. These results indicate the equipotential vasodilatory effect of CGRP on pulmonary and systemic vessels. In view of the similar hemodynamic changes after CGRP and acetylcholine, it is interesting to consider that the mechanisms of action also might be similar. Acetylcholine acts through nitric oxide release (33), and recent studies strongly suggest a similar role for nitric oxide in the vasodilatory action of CGRP (1, 16). However, other mechanisms, such as KATP channel

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Table 2. Hemodynamic effects of single injections of CGRP or acetylcholine

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<tr>
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<th>CGRP</th>
<th>Acetylcholine</th>
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<tr>
<td>LPA blood flow, ml/min</td>
<td>26 ± 22</td>
<td>202 ± 86*</td>
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<tr>
<td>Mean pulmonary arterial blood pressure, mmHg</td>
<td>58 ± 5</td>
<td>48 ± 6*</td>
</tr>
<tr>
<td>Mean ascending aortic blood pressure, mmHg</td>
<td>56 ± 3</td>
<td>46 ± 5*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>182 ± 16</td>
<td>197 ± 36</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>LPA resistance, mmHg·min·ml⁻¹</td>
<td>3.69 ± 2.57</td>
<td>0.24 ± 0.12*</td>
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</table>

Values are means ± SD. CGRP, calcitonin gene-related peptide; LPA, left pulmonary artery. *P < 0.05 comparing baseline with maximum.
activation or adenosine 3',5'-cyclic monophosphate generation (22, 30) also have been suggested, and further investigation is needed to determine the exact mechanisms by which CGRP produces pulmonary vasodilatation in the fetus.

With repeated CGRP injections, although left pulmonary arterial blood flow returned to baseline 5 min after the first injection of CGRP (Fig. 1), repeated injections every 5 min significantly increased left pulmonary arterial blood flow in a stepwise manner (Fig. 3A). Likewise, left pulmonary arterial resistance returned to baseline within 5 min after the first injection (Table 2), but then decreased progressively with repeated injections (Fig. 3F). To explain these phenomena, we speculate that there may be specific and different functions of CGRP receptors associated with the accumulating plasma concentration of CGRP, e.g., at least two different types of CGRP receptor with different affinities. When plasma CGRP concentrations are relatively low, the effect could be mediated by a receptor with high affinity, but a short duration of action. When concentrations exceed a certain level, another CGRP receptor could be activated with a lower affinity, and with a more prolonged response. Alternatively, different mechanisms of achieving a prolonged increase in pulmonary arterial blood flow could be invoked; further study of these potential mechanisms is necessary.

Previous in vivo studies have demonstrated a positive chronotropic effect of CGRP (9, 12, 13), but the responsible mechanism remains controversial. Some investigators suggest that an increase in heart rate after CGRP administration could be secondary to the decrease in systemic blood pressure (9, 13); others have revealed that CGRP-rich fibers extend to the nodal and conductive systems, near the pacemaker cells in the heart, suggesting that CGRP has a primary effect (10).

**Fig. 2.** Example of stepwise increase in LPA blood flow after 5 sequential bolus injections of CGRP given at 5-min intervals. LPA blood flow was assessed every 5 s during 5 min after each injection. ●, First injection; ○, 2nd injection 5 min after 1st; □, 3rd injection 10 min after 1st; △, 4th injection 15 min after 1st; ▲, 5th injection 20 min after 1st.

**Fig. 3.** Regression of time to LPA flow (A), mean pulmonary arterial blood pressure (B), mean ascending aortic blood pressure (C), heart rate (D), left atrial pressure (E), and LPA resistance (F) with repeated CGRP injections. MPA, main pulmonary artery; NS, no statistical significance.
In this study, repeated CGRP injections increased heart rate progressively (Fig. 3C), but with no significant decrease in systemic arterial blood pressure (Fig. 3D). These data support a primary, positive chronotropic effect of CGRP.

In a similarly designed recent study, we demonstrated, in fetal sheep, a pulmonary vasodilatory effect of adrenomedullin, a recently discovered potent vasodilatory peptide with a structure partly homologous to CGRP (8). Both peptides decreased mean pulmonary arterial blood pressure and increased left pulmonary arterial blood flow nearly 10-fold from baseline. Interestingly, however, there are two apparent differences in the hemodynamic changes. First, 5 min after the first injection, pulmonary arterial blood flow returned to baseline values after the peak increase with CGRP (Fig. 1), but it remained significantly higher than baseline after adrenomedullin. Second, with repeated injections, heart rate increased progressively with CGRP but did not change significantly with adrenomedullin. Consequently, these data indicate that CGRP has a less prolonged action than adrenomedullin in fetal sheep and also is chronotropic.

Blood gas analysis showed significantly increased PCO2 and lactate and decreased pH after the experiments (Table 1). Often in the fetal circulation, a continuous increase in pulmonary blood flow leads to a decrease in blood flow through ductus arteriosus, and resultant decreased perfusion of postductal areas supplied by the descending aorta. Therefore, the blood gas changes we observed could be explained by metabolic responses to poor perfusion of the postductal areas (including the placenta). Although not statistically significant, there was an almost 12% decrease in hemoglobin concentration after the experiments (P = 0.06). Some investigators have reported a relationship between serum CGRP concentration and fluid excess in humans, e.g., an increase in serum CGRP concentrations in patients with chronic heart failure and during the ultrafiltration phase of hemodialysis (27). Interestingly, after adrenomedullin infusion, there was a significant decrease in hemoglobin concentrations, also by an unknown mechanism (10). Further study is required of the effects of CGRP, as well as adrenomedullin, on fluid homeostasis.

In conclusion, exogenously administered CGRP significantly decreases pulmonary vascular resistance and increases pulmonary arterial blood flow in fetal sheep. This finding suggests that endogenous CGRP may play a role in the remarkable decrease in pulmonary vascular resistance and increase in pulmonary arterial blood flow during the transition from fetal to extraterine life. Further study, therefore, is necessary to fully evaluate the possible physiological role of CGRP in the perinatal period and also the mechanisms by which CGRP produces its effects.

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