Cortisol pretreatment enhances the lung growth response to tracheal obstruction in fetal sheep

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Boland, Rochelle E., Laura Nardo, and Stuart B. Hooper. Cortisol pretreatment enhances the lung growth response to tracheal obstruction in fetal sheep. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L1126–L1131, 1997.—We have investigated whether cortisol pretreatment of sheep fetuses will result in a greater liquid accumulation within the lung and a greater lung growth response to obstruction of the fetal trachea. Chronically catheterized fetal sheep received either 1) a cortisol infusion at an increasing dose (1.5–4.0 mg/day) from days 118 to 127 of gestation; the fetal trachea was then obstructed from days 128 to 131 of gestation (n = 4); 2) a saline infusion from days 118 to 127 of gestation with no period of tracheal obstruction (control; n = 4); or 3) a saline infusion from days 118 to 127 of gestation, followed by obstruction of the trachea (n = 4). The saline infusion was started on day 121 of gestation, and the fetal lung liquid secretion rates and volumes were measured on days 118, 128, and 131 of gestation. On day 131 of gestation, all fetuses were given an intravenous injection of [3H]thymidine and were killed 8 h later. Cortisol pretreatment increased the volume of liquid that accumulated within the fetal lung. The fetal lung liquid volume increased by 1040 mg/kg between days 118 and 127 of gestation, 30% of this increase occurred within 5 days. In addition, we found that cortisol pretreatment significantly enhanced the fetal lung growth rate, with volume reaching 14.1 ml/kg after 3 days of tracheal obstruction. We conclude that cortisol pretreatment increases the fetal lung growth response to tracheal obstruction.

MATERIALS AND METHODS

Experimental design. The fetuses were randomly assigned to one of three different groups after surgery. One group of fetuses received a 9-day saline infusion (control; n = 4). The saline infusion was started on day 121 of gestation, and the fetal lung liquid secretion rates and volumes were measured on days 118, 128, and 131 of gestation. On day 131 of gestation, all fetuses were given an intravenous injection of [3H]thymidine and were killed 8 h later. Cortisol pretreatment increased the volume of liquid that accumulated within the fetal lung. The fetal lung liquid volume increased by 1040 mg/kg between days 118 and 127 of gestation, 30% of this increase occurred within 5 days. In addition, we found that cortisol pretreatment significantly enhanced the fetal lung growth rate, with volume reaching 14.1 ml/kg after 3 days of tracheal obstruction. We conclude that cortisol pretreatment increases the fetal lung growth response to tracheal obstruction.

Surgical procedure. Surgery was performed on 12 Merino × Border Leicester ewes at 112 ± 1 days of gestation. Two large-bore Silastic catheters (Dow Corning) were inserted into the fetal trachea: one was directed toward the fetal lung lumen and the other was directed toward the uterus. The catheters were connected externally to form an exteriorized tracheal loop. Polyvinyl catheters (Dow Plastics) were also implanted in a fetal carotid artery and jugular vein and the amniotic sac. Antibiotics were administered to the fetus [2 ml im, 200 mg/ml procaine penicillin (Intervet)] before it was returned to the uterus. The catheters were exteriorized through an incision in the right flank of the ewe. The animals were allowed at least 5 days to recover before experiments began. Fetal arterial blood was collected daily to measure pH, arterial Pco2, arterial Po2, and arterial O2 saturation with an ABL510 blood gas and acid-base analyzer (Radiometer) to assess fetal well-being.

Experimental design. The fetuses were randomly assigned to one of three different groups after surgery. One group of fetuses received a 9-day saline infusion. A second group of fetuses received a 9-day saline infusion.
(118–127 days of gestation), and after an intervening period of 24 h, the fetal trachea was obstructed for 3 days (128–131 days of gestation; group O). The third group of fetuses was a control group that received a 9-day saline infusion (118–127 days of gestation), but the trachea was not obstructed (group C).

The cortisol solution was prepared daily in heparinized saline; the doses of cortisol (hydrocortisone sodium succinate: Solu Cortef, Upjohn) were 1.5 mg/day on days 118–119, 2.5 mg/day on days 120–121, 3.0 mg/day on days 122–123, 3.5 mg/day on days 124–125, and 4.0 mg/day on days 126–127. This regimen was similar to that used by Wallace et al. (21) and does not affect fetal lung growth. Our aim was to prematurely increase fetal plasma cortisol concentrations in a manner similar to that observed over the last 10 days of gestation in fetal sheep without inducing labor (13). Groups C and O were infused with equal volumes of heparinized saline that were delivered at the same rate (1.2 ml/h) as in the cortisol-infused fetuses. All infusions were stopped on day 127 of gestation so that circulating fetal cortisol concentrations had 24 h to return to control levels before the trachea was obstructed. The trachea was obstructed by blocking the exteriorized tracheal loop, which prevents the normal flow of liquid in and out of the lungs. The period of obstruction began on day 128 of gestation and continued for 3 days (until day 131) in two groups of fetuses (groups O and F); the flow of liquid in and out of the lungs was not obstructed in the other group of fetuses (group C).

The volumes and secretion rates of fetal lung liquid were measured on days 118 (before start of the infusions), 128 (before obstruction of the trachea in groups F and O), and 131 of gestation (immediately before postmortem examination). This measurement was made with an indicator dilution technique (7, 19). Tracheal pressures were measured with pressure transducers (DTX transducers, Ohmeda) and were displayed on a polygraph recorder after electronic subtraction of the amniotic sac pressure. Measurements were made at 1-min intervals during apnea over a 6-h period on days 128 (before obstruction) and 129–131 of gestation. The mean pressure of each 6-h block was then calculated for comparison among the different treatment groups.

Fetal blood samples were collected every second day for the determination of fetal plasma cortisol concentrations. The radioimmunoassay used to measure cortisol concentrations was identical to that described by Bocking et al. (2).

Eight hours before the ewe and fetus were killed, the fetus was given a bolus injection of [3H]thymidine (1 mCi/kg; ICN Pharmaceuticals) into the fetal jugular vein. The fetal lungs were drained of liquid immediately before the ewe and fetus were killed; this was achieved by administering an overdose of pentobarbital sodium (130 mg/kg iv) to the ewe. The fetus was removed and weighed, and the fetal heart, lungs, kidneys, and liver were dissected free and then weighed. Portions of the left lung, liver, and kidneys were frozen in liquid nitrogen and stored at −70°C for later biochemical analysis. Total lung DNA and protein contents and the incorporation of [3H]thymidine into DNA were measured with previously described methods (8).

Statistical analysis. Fetal plasma cortisol concentrations, tracheal pressures, and fetal lung liquid volumes and secretion rates were measured with a two-way analysis of variance for repeated measures, with the factors being animals, groups, and time. When significant differences occurred (P < 0.05), the differences between sample times and groups were analyzed with the Student-Newman-Keuls test. Differences between treatment groups in fetal organ weights, organ-to-body weight ratios, DNA content, protein content, and [3H]thymidine incorporation into DNA were all tested with a one-way analysis of variance. Results are expressed as means ± SE.

RESULTS

Fate of fetuses and fetal body weights. Fetal body weights measured immediately postmortem were not significantly different among the different groups (group C, 3.52 ± 0.48 kg; group O, 3.68 ± 0.53 kg; group F, 3.50 ± 0.42 kg). Mean arterial fetal blood gas values were not significantly different among the three groups of fetuses. Mean values for all animals during the experiments were pH 7.366 ± 0.003, arterial P O2 49.8 ± 0.5 mmHg, arterial P O2 21.2 ± 0.9 mmHg, and arterial O2 saturation 64.3 ± 2.3%, which were within the normal ranges of values for fetuses of similar gestational ages in our laboratory.

Fetal plasma cortisol concentrations. Before the infusion of cortisol, plasma cortisol concentrations were similar in all three groups of fetuses (group C, 1.6 ± 1.0 ng/ml; group O, 1.5 ± 0.5 ng/ml; group F, 1.2 ± 0.6 ng/ml). Mean plasma cortisol concentrations for the cortisol-infused fetuses (group F) were significantly greater than the concentrations measured in both groups of saline-infused fetuses during the infusion period; plasma cortisol concentrations increased to 28.9 ± 6.7 ng/ml on day 127 of gestation. Within 1 day of stopping the cortisol infusion, fetal plasma cortisol concentrations had returned to control values (Fig. 1).

Fetal tracheal pressure changes. On day 128 of gestation, after the completion of the infusion period, tracheal pressures measured in all fetuses were similar (group C, 1.4 ± 0.1 mmHg; group O, 1.3 ± 0.2 mmHg; group F, 1.3 ± 0.1 mmHg). Hatched bar, infusion period (days 118–127 of gestation); solid bar, period of tracheal obstruction (days 128–131 of gestation). *Significantly greater than control values.

Fig. 1. Fetal plasma cortisol concentrations measured in control fetuses (saline infused, no tracheal obstruction; ●), saline-infused tracheal obstructed fetuses (■), and cortisol-infused tracheal obstructed fetuses (▲).
group of fetuses; Fig. 2). After obstruction of the fetal trachea, tracheal pressures increased to 5.1 ± 0.7 mmHg in the saline-infused fetuses and to 5.0 ± 0.3 mmHg in the cortisol-infused fetuses within 1 day. These values were significantly greater than the control values but were not significantly different from each other. The tracheal pressures remained at these elevated levels for the duration of the obstruction period (Fig. 2).

Fetal lung liquid volumes. Measured on days 118 and 128 of gestation, lung liquid volumes were similar in all groups of fetuses (Fig. 3). However, on day 131 of gestation, the volume of lung liquid measured in the saline-infused fetuses after 3 days of tracheal obstruction (group O, 69.5 ± 4.1 ml/kg) was significantly greater than that measured in the control fetuses (group C, 37.2 ± 3.2 ml/kg). The volume of lung liquid measured in the cortisol-infused fetuses at 131 days, after 3 days of tracheal obstruction (group F 96.1 ± 14.1 ml/kg), was significantly greater than that measured in both other groups of fetuses.

Fetal lung liquid secretion rates. Lung liquid secretion rates measured on day 118 of gestation were similar in all groups of fetuses. However, on day 128 of gestation, lung liquid secretion rates measured in the cortisol-infused fetuses (4.9 ± 0.7 ml·h⁻¹·kg⁻¹) were significantly greater than those measured in the control fetuses (2.6 ± 0.8 ml·h⁻¹·kg⁻¹). On the final day of the experimental period (day 131 of gestation), fetal lung liquid secretion rates had decreased to undetectable levels in the two groups of fetuses exposed to tracheal obstruction (groups O and F; Fig. 3).

Fetal organ weights. When corrected for fetal body weight, lung weights in the saline-infused fetuses after 3 days of tracheal obstruction were significantly greater than those of the control fetuses (Table 1). However, fetuses that received cortisol infusions before the tracheal obstruction had lung weights that were significantly greater than both the control and saline-infused tracheal-obstructed fetuses (Table 1). Fetal heart and kidney weights were not significantly different among the groups. Fetal liver weights were not different between the saline-infused groups of fetuses (Table 1). However, compared with the control fetuses (group C), the cortisol-infused fetuses had significantly reduced liver weights, although this reduction was not quite significant when compared with the saline-infused tracheal-obstructed fetuses.

DNA content, protein content, and DNA synthesis rates. In the saline-infused fetuses, 3 days of tracheal obstruction significantly increased the total lung DNA
content from 182.2 ± 5.2 (group C) to 257.4 ± 11.0 mg/kg (group O). The total DNA content of lungs from the cortisol-infused tracheal-obstructed fetuses (309.1 ± 16.3 mg/kg) was significantly greater than in both the control and saline-infused tracheal-obstructed fetuses (Fig. 4). The DNA content of the livers and kidneys for each group of animals was not significantly different.

Pulmonary DNA synthesis rates, as measured by the incorporation of [3H]thymidine into DNA, were similar in the cortisol-infused [225.6 ± 54.0 disintegrations·min⁻¹ (dpm)·mg DNA⁻¹] and saline-infused [213.5 ± 26.0 dpm/mg DNA] fetuses after 3 days of tracheal obstruction; both of these values were significantly greater than the DNA synthesis rates measured in the control fetuses [86.4 ± 26.7 dpm/mg DNA; Fig. 4]. No significant differences were found in the DNA synthesis rates measured in the fetal liver or kidneys.

Total lung protein content was similar in the cortisol-infused and saline-infused fetuses after 3 days of tracheal obstruction, although both of these values were significantly greater than the total lung protein content measured in the control fetuses (Table 1).

### DISCUSSION

Our findings demonstrate that pretreatment of fetuses with a 9-day cortisol infusion increases the volume of liquid that accumulates within the lung after 3 days of tracheal obstruction and enhances the increase in lung DNA content. We propose that, in response to fetal tracheal obstruction, the enhanced lung growth response and lung liquid accumulation induced by cortisol pretreatment are related. That is, the enhanced lung growth response to tracheal obstruction most probably results from the enhanced accumulation of lung liquid within the future airways in cortisol-pretreated fetuses.

The increase in lung liquid volume we observed after 3 days of tracheal obstruction in cortisol-infused fetuses (96.1 ± 14.1 ml/kg) was greater than that observed in saline-infused fetuses and was similar to the value achieved after 7 days of tracheal obstruction in the absence of cortisol (97.3 ± 15.2 ml/kg) (see Ref. 18).

This indicates that cortisol enhances the retention of lung liquid after tracheal obstruction. Possible explanations for the greater increase in lung liquid volume in cortisol-infused fetuses include an increase in lung tissue compliance or an increase in the secretion rate of lung liquid. A greater increase in intraluminal pressure cannot readily explain the difference in lung liquid volume because the increase in pressure was similar in both saline-infused and cortisol-infused fetuses after tracheal obstruction (Fig. 2). We suggest that the increase in lung liquid volume resulted from an increase in lung tissue compliance; this would allow a greater accumulation of lung liquid for a given increase in intraluminal pressure. Indeed, the administration of cortisol to fetuses is known to increase compliance of both air-filled (10, 11) and liquid-filled (5, 15) lungs that may result, in part, from changes in the lung's extracellular matrix (20). Indeed, cortisol infusions induce marked changes in the lung ultrastructure, leading to reduced interalveolar distances and increased surface areas (4).

### Table 1. Wet lung and liver weights and total lung protein content in control fetuses and in saline- and cortisol-infused fetuses after 3 days of tracheal obstruction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet Lung Weight, g/kg</th>
<th>Wet Liver Weight, g/kg</th>
<th>Total Lung Protein Content, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline infused, no tracheal obstruction</td>
<td>34.1 ± 1.5</td>
<td>29.0 ± 1.9</td>
<td>2,548.5 ± 49.7</td>
</tr>
<tr>
<td>Saline infused plus tracheal obstruction</td>
<td>49.4 ± 1.9*</td>
<td>25.3 ± 1.9</td>
<td>3,655.5 ± 249.9*</td>
</tr>
<tr>
<td>Cortisol infused plus tracheal obstruction</td>
<td>54.3 ± 1.3†</td>
<td>19.2 ± 2.5*</td>
<td>3,809.5 ± 425.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from control fetuses (saline infused, no tracheal obstruction). †Significantly different from saline-infused tracheal-obstructed fetuses.
Total lung DNA content had increased to a value that was 44% greater than the control value after only 3 days of tracheal obstruction, which confirms previous findings from our laboratory (8, 12, 18) that short periods of tracheal obstruction are a potent stimulus for fetal lung growth. On the other hand, cortisol pretreatment enhanced the increase in lung DNA content induced by 3 days of tracheal obstruction to a value that was 68.9% greater than in control fetuses. Previously, it has been shown that the total lung DNA content increases to a maximum value of 60–70% above the control value by day 7 after the fetal trachea was obstructed (8, 18). Thus cortisol pretreatment enhanced the lung growth response to tracheal obstruction such that the maximum increase in DNA content was reached in 3 days rather than in 7 days. It is unlikely that the accelerated lung growth would have proceeded beyond this value because further growth is probably restricted by the physical limitations imposed by the chest wall (8, 18), which is unlikely to be affected by cortisol treatment.

It has previously been shown that the increase in lung growth after tracheal obstruction is closely related (r = 0.99) to the increase in lung liquid volume and not to the increase in intraluminal pressure (18). This suggests that the increase in lung liquid volume is the primary determinant for the increase in lung growth after tracheal obstruction. Our findings that cortisol pretreatment enhanced both the lung liquid accumulation and the lung growth caused by tracheal obstruction in the absence of a greater increase in the intraluminal pressure are consistent with this suggestion. This contention has significant implications for understanding the mechanisms by which increases in lung expansion stimulate fetal lung growth. For instance, the increase in lung growth after tracheal obstruction must depend primarily on an increase in lung volume. This could indicate that the mitogenic response of individual cells is primarily dependent on the degree to which the cells are physically extended. In addition, all available evidence indicates that the extent of the increase in lung volume is a good indicator of the lung growth that has occurred after tracheal obstruction (18). This knowledge may be of benefit if tracheal obstruction is to be used therapeutically in human fetuses to reverse fetal lung growth deficits.

The increases in pulmonary DNA synthesis rates over the last 8 h of the 3-day tracheal obstruction period were not different between the saline- and cortisol-infused fetuses. Under normal conditions, the increase in DNA synthesis rates induced by tracheal obstruction is maximal at 2 days, is reduced but still elevated at 4 and 7 days, and has returned to control values after 10 days of tracheal obstruction (18). The increase in DNA synthesis measured in the saline-infused fetuses after 3 days of tracheal obstruction (147% above control values) was less than the increase observed after 2 days (777%) (12) but is greater than the value measured after 4 days (66%) of tracheal obstruction (18). These findings provide further evidence indicating that DNA synthesis rates increase to a very high level initially but then gradually return to control values after 10 days. It is not clear why the measured DNA synthesis rates were similar in saline-infused and cortisol-infused fetuses, given the large difference in DNA content. However, it is possible that either 1) the DNA synthesis rate peak was higher in cortisol-treated fetuses and had returned to the levels observed in saline-infused fetuses by the time the 8-h measurement period had commenced or 2) peak synthesis rates occurred earlier and had returned to the level observed in saline-infused fetuses by the third day after tracheal obstruction.

The increases in total lung protein content induced by 3 days of tracheal obstruction were similar in saline- and cortisol-infused fetuses. This result is not surprising considering that the increase in total lung protein content reaches a maximum after 2 days of tracheal obstruction (12, 18). Thus it is likely that after 3 days of tracheal obstruction the increase in total protein content would have reached maximal values in both the saline- and cortisol-infused fetuses when the measurements were made. Consequently, it is not known whether cortisol pretreatment accelerates the rate of increase in protein content because much shorter periods of tracheal obstruction would be required to detect such differences.

The finding that lung liquid production rates were significantly greater in the cortisol-infused compared with the saline-infused fetuses at the end of the infusion period before the trachea was obstructed is consistent with the previous findings of Wallace et al. (21, 22). These data indicate that cortisol may act directly or indirectly to affect the lung liquid secretory mechanism, although the mechanisms involved are unknown. Wallace et al. suggested that cortisol may act to increase some or all of the cellular components responsible for the secretion of fetal lung liquid. Alternatively, cortisol may increase the luminal surface area and, therefore, increase the area across which liquid can be secreted. However, lung liquid production rates were reduced to undetectable levels after tracheal obstruction, which is consistent with the previous observations of Nardo et al. (17, 18). This indicates that the positive effects of cortisol on fetal lung liquid production are negated by obstructing the trachea. Miller et al. (14) and Nardo et al. (18) suggested that the cessation of lung liquid secretion in response to tracheal obstruction results from an associated increase in hydrostatic pressure within the lung lumen that counteracts the osmotic pressure driving lung liquid secretion.

In the present study, a 9-day cortisol infusion did not significantly increase fetal lung liquid volumes, which is not consistent with the previous findings of Wallace et al. (21). The difference between the findings of this study and those of the previous study (21) could be due to a lower number of fetuses used in the present study (n = 4 vs. n = 6) or perhaps to the timing of the lung liquid volume measurements. In the present study, these were made 24 h after the cortisol infusions had ceased.
This study has shown that prolonged cortisol infusions can be used to enhance the lung growth response to tracheal obstruction without inducing labor. Cortisol pretreatment also enhanced the increase in lung liquid accumulation after tracheal obstruction in the absence of a greater increase in tracheal pressure. This indicates that the greater increase in lung liquid volumes in cortisol-infused fetuses may have resulted from an associated increase in lung tissue compliance. We suggest that the greater lung growth response in cortisol-infused fetuses results from the retention of a greater volume of lung liquid after tracheal obstruction, which provides a greater stimulus for lung growth.

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