Postnatal Age-Related Renal Responses to Hypoxemia in Lambs

DOUGLAS N. WEISMANN AND WILLIAM R. CLARKE

SUMMARY The role of normocapnic hypoxemia (arterial PaO₂ 33 ± 7 torr for 30 minutes) in asphyxial renal failure and its modification by maturation of renal function was studied in 50 chronically catheterized, unanesthetized lambs of 2-38 days postnatal age. Arterial pH and PaCO₂ did not change significantly in response to hypoxemia in these lambs. Normocapnic hypoxemia was associated with (1) significant percent increases in arterial serum osmolality (1.82 ± 2.96%, P = 0.0001), arterial blood lactate concentration (1009 ± 2092%, P = 0.0018), arterial blood hematocrit (6 ± 12%, P = 0.0016), arterial hemoglobin concentration (4.8 ± 6.5%, P = 0.0004), arterial plasma vasopressin (2370 ± 3346%, P = 0.0001), arterial plasma renin activity (153 ± 230%, P = 0.0001), arterial plasma aldosterone (91.2 ± 143%, P = 0.0001), and fractional sodium excretion rate (120 ± 240%, P = 0.007); and (2) significant percent decline in glomerular filtration rate (-22.6 ± 32.6%, P = 0.0003). Several responses to hypoxemia correlated significantly with postnatal age, including (1) positive correlation of postnatal age with percent change in blood osmolality (r = 0.36, P = 0.010), hematocrit (r = 0.48, P = 0.0005), hemoglobin (r = 0.59, P = 0.0004), and lactate (r = 0.73, P = 0.0001), suggesting greater water movement from the intravascular compartment in response to hypoxemia in more mature lambs; and (2) positive correlation of postnatal age with percent change in urinary flow rate (r = 0.66, P = 0.0001), urinary sodium excretion rate (r = 0.65, P = 0.0001), and osmolar clearance rate (r = 0.60, P = 0.0002), suggesting a greater effect of hypoxemia on the renal tubules to decrease sodium reabsorption in more mature kidneys. Thus, normocapnic hypoxemia may play a role in asphyxial renal failure, and the immature kidney does not have increased susceptibility to this condition. Circ Res 49:1332-1338, 1981

HYPOXEMIA associated with respiratory disease or birth asphyxia is encountered frequently in newborns. A reversible decrease in renal function (Guignon et al., 1975) and renal failure (Dauber et al., 1976) has been documented under these conditions. The role of hypoxemia, in the absence of hypercarbia and acidosis, in these newborn renal responses is not clear. Furthermore, it is not known whether the newborn kidney is more susceptible to these adverse conditions relative to older subjects. Indeed, variation in renal pathophysiologic responses with age may be expected, since renal function is immature at birth and undergoes a rapid maturation during the first weeks of life (Aperia et al., 1974; Fawer et al., 1979). The present study was undertaken to examine the effect of this early postnatal maturation of renal function on renal responses to hypoxemia. The following questions were addressed: (1) does hypoxemia play a role in asphyxial renal failure of the newborn? and (2) is the functionally immature kidney of the newborn more susceptible to hypoxemia? The responses to hypoxemia were studied in a chronically catheterized lamb experimental model in the absence of effects of surgical stress, anesthesia, and changes in pH and PaCO₂. Responses of renal hemodynamics, filtration rates, and excretion rates to hypoxemia were examined simultaneously with factors which may modulate these responses in the maturing animal, including plasma renin activity, plasma aldosterone, plasma arginine vasopressin, and urinary excretion of prostaglandin E.

Methods

Mixed-breed Dorset-Suffolk lambs were housed with the maternal ewe in the University Animal Care Unit. Lambs were selected for experimental procedures according to their postnatal age.

Surgical Procedures

Anesthesia was induced with thiopental, 10 mg/kg, administered intravenously. Orotracheal intubation was performed and each animal was ventilated with a mixture of 30% oxygen and 0.5% halothane by means of a Byrd respirator and a standard anesthesia cart. Body temperature was maintained with a thermostatically controlled circulating water-bath heating pad.

The lamb's abdomen and left flank were prep and draped in a sterile fashion and all subsequent surgical procedures were performed utilizing sterile technique. The right femoral artery was cannulated with PE tubing, and the catheter tip was advanced...
to the left ventricular cardiac chamber. Correct placement of the catheter tip was documented by the recording (Beckman R611 Dynograph) of a characteristic intraventricular pressure curve (Statham pressure transducer). The catheter then was secured with silk ties at the level of the femoral artery. The ipsilateral femoral vein and contralateral femoral artery also were catheterized with catheter tip placement at the bifurcation of the distal inferior vena cava and the distal abdominal aorta, respectively. These catheters were also secured with silk ties. Catheter tip placement was verified by direct observation when the animals were killed.

Through a suprapubic incision, a PE 180 tube was placed in the urinary bladder and secured with a purse-string tie with chromic suture. All cannulas were tunneled subcutaneously to exit the skin at the left flank. Incisions were closed with 3-0 chromic purse-string tie with chromic suture. All cannulas were tunnelled subcutaneously to exit the skin at the left flank. Incisions were closed with 3-0 chromic suture. The lambs were allowed 48-72 hours for recovery from the surgical procedures. The lambs were administered glucose-saline solution intravenously postoperatively until they were able to stand and feed by mouth. Thereafter, the lamb nursed from the ewe ad libitum. The lambs received ampicillin, 200 mg/kg intravenously, every 12 hours until studied.

**Experimental Protocol**

Lambs were studied across a range of postnatal age (2–38 days) over which rapid increases in renal function occur (Aperia et al., 1974). Studies of these chronically catheterized, unanesthetized lambs were performed while the lambs were supported in a standing posture by a specially designed canvas harness. The lambs, in general, remained quiet under these conditions. Heart rate (HR) and arterial blood pressure (MAP) were recorded continuously on a Beckman R611 Dynograph by means of a pressure transducer (Statham) connected to the femoral artery catheter. In each lamb, a 2-4 μCi loading dose of 125I-sodium iohalamate (Abbott Laboratories) in a 5% dextrose in water solution was administered intravenously, followed by a constant infusion of 125I-sodium iohalamate in a 5% dextrose in water solution at a rate of 0.05 to 0.1 ml/min, using a Harvard withdrawal infusion pump. This infusion rate delivered 0.05 to 0.1 μCi of 125I-sodium iohalamate per minute.

After a 60-minute equilibration period, three baseline (control) clearance periods of 20 minutes each were performed. Urine was collected quantitatively. At the midpoint of each period, arterial blood was sampled for determinations of 125I-iohalamate content, sodium concentration (Na), potassium concentration (K), chloride concentration (Cl), osmolality (Osm), hematocrit (Hct), hemoglobin concentration (Hb), oxyhemoglobin saturation (HbO2), pH, PO2, and Pco2. During the third baseline clearance period, blood samples were drawn also for aldosterone concentration (Aldo), vasopressin concentration (AVP), lactic acid concentration (Lact), and plasma renin activity (PRA). Withdrawn blood was volumetrically replaced with plasmanate. After completion of the third baseline clearance period, approximately 1.5 x 106 microspheres (3M Company) labeled with a single radioactive isotope (Ce, Sr, Sc, or Nb) were suspended in 3 ml of 0.9% saline, agitated thoroughly, and injected into the left ventricular catheter over 20-30 seconds, then flushed immediately with 5 ml of 0.9% saline solution. An independent lower body reference sample was obtained by withdrawal of blood (Harvard infusion withdrawal pump) through the left femoral arterial catheter at a rate of 1.94 ml/min for a period of 3 minutes starting approximately 20 seconds before the microspheres injection (Makowski et al., 1968). Subsequently, systemic hypoxemia was produced by directing a 12% oxygen in nitrogen gas mixture into a clear plastic bag which was placed over the animal’s head and secured with a drawstring at the neck. This procedure compelled the lamb to partially rebreathe expired gases containing CO2, within the bag enclosing the head, so that hyperpnea produced by the hypoxemia would not induce hypocarbia. Approximately 20 minutes after initiation of the oxygen-deficient inhaled gas mixture, systemic hypoxemia was confirmed by arterial blood gas analysis, and a 10-minute clearance period was performed. Urine was, again, collected quantitatively. At the midpoint of the clearance period, blood was drawn for determinations as in the third baseline period. After the 10-minute clearance period during hypoxemia, an injection of radioactive microspheres labeled with a different isotope was performed as previously described. The animal then was killed by intravenous administration of saturated KCl solution. The kidneys were immediately harvested, weighed, cut into sagittal sections of approximately 1 g, and placed in counting vials.

**Analytical Procedures**

The concentrations of sodium (mEq/liter) and potassium (mEq/liter) in blood and urine were measured with a flame photometer. Chloride (mEq/liter) in blood and urine was measured by potentiometric titration (Radiometer MT10 chloride titrator). Blood and urine osmolality (mOsm/Kg H2O) were determined by freezing point depression (Advanced Instruments osmometer). Arterial blood pH and gases (PO2, PCO2 expressed as torr) were determined by a Radiometer pH/blood gas analyzer. Hemoglobin (g/dl) and oxyhemoglobin saturation (%) were determined by an IL Cooximeter. 125I-iohalamate in blood and urine, and radio-labeled microspheres (Sr, Ce, Sc, Nb) in blood and tissue, were counted in a γ spectrometer (Beckman Instruments). Plasma renin activity (ng angiotensin/ml per hour) was determined by the method of Haber et al. (1969) as modified by Oparil (1975),
using radioimmunoassay of angiotensin I generated in vitro from the animal’s plasma under controlled conditions. Lactate content of blood (mg/dl) was determined by the method of Noll (1974). Plasma aldosterone (Aldo, expressed in pg/ml) (Robillard et al., 1980), plasma vasopressin (AVP, expressed in μU/ml) (Skowsky et al., 1974), and urinary prostaglandin E (PGE, expressed in ng/ml) (Van Orden et al., 1977) measurements were performed by radioimmunoassay in the Cardiovascular Center Core Laboratory at the University of Iowa. Hematocrit (%) was determined by standard micro methods.

Calculations

Total renal blood flow (TRBF) was determined according to the following formula: TRBF (ml/min) = total kidney counts × femoral artery reference flow/total femoral blood counts. Renal vascular resistance (RVR) was determined according to the formula RVR (mm Hg/ml per min) = RPP/RBF, where RPP is the renal perfusion pressure estimated to be equal to aortic pressure minus the inferior vena cava pressure expressed in mm Hg and RBF is the renal blood flow expressed in ml/min. Glomerular filtration rate (GFR) was estimated from the clearance of 125I-sodium iothalamate. Osmolar clearance rate was determined according to the formula Cosm (mOsm/min) = (Uosm/V)/Posm, where Uosm and Posm are urine and plasma osmolality, respectively, in mOsm/kg H2O, and V is the urinary flow rate in ml/min. Urinary excretion rates of sodium (UNaV), chloride (UCrV), and potassium (UKrV) were calculated and expressed as micro equivalents per minute. Urinary excretion rate of prostaglandin E (UPGEV) was calculated and expressed as nanograms per minute. Fractional excretion of sodium (FENa) was calculated and expressed as percent.

Data Analysis

Comparisons of baseline and hypoxemia values were done by Student’s paired t-test. Correlation coefficients and linear regressions were computed by least squares formulas (Steel and Torrie, 1960). A P value of 0.01 or less was required for a difference or a correlation to be declared significant.

Table 1 Arterial Blood Values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>P02 (torr)</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Oxyhemoglobin (%sat.)</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Pco2 (torr)</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>pH</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Sodium (mEq/liter)</td>
<td>147 ± 4</td>
<td>147 ± 4</td>
</tr>
<tr>
<td>Potassium (mEq/liter)</td>
<td>4.0 ± 0.4</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>Chloride (mEq/liter)</td>
<td>111 ± 5</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg H2O)</td>
<td>296 ± 9</td>
<td>300 ± 11*</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = number of lambs.

* Value significantly different from baseline, P = 0.0001.

Figure 1

The percent change in arterial lactic acid concentration in response to hypoxemia (ΔLact) in relation to postnatal age; n = 47, r = 0.72, P = 0.0001.

Figure 2

a: The percent change in arterial hematocrit in response to hypoxemia (ΔHct) in relation to postnatal age; n = 49, r = 0.48, P = 0.0005. b: The percent change in arterial hemoglobin concentration in response to hypoxemia (ΔHb) in relation to postnatal age; n = 32, r = 0.59, P = 0.0004.
Results

Measurements performed during the three baseline periods were averaged for each lamb. Baseline and hypoxemia arterial blood values are recorded in Table 1. The administration of low oxygen inhaled gas mixture produced the desired significant declines in arterial Po2 and oxyhemoglobin saturation. The change with hypoxemia of arterial pH, pCO2, sodium, potassium, and chloride was not significant, and neither baseline nor change in these variables with hypoxemia correlated significantly (P > 0.04) with postnatal age. On the other hand, serum osmolality increased significantly in response to hypoxemia (1.82 ± 2.96%, P = 0.001) and the percent change in serum osmolality with hypoxemia (ΔOsm) correlated positively with postnatal age (r = 0.36, P = 0.010). A similar response was seen in the percent increase in arterial lactate concentration with hypoxemia (ΔLactate 1009 ± 2092%, P = 0.0018) which also correlated with postnatal age (Fig. 1). Likewise, the increase in arterial blood hematocrit with hypoxemia (ΔHct 6 ± 12%, P = 0.0016) and the increase in arterial hemoglobin concentration with hypoxemia (ΔHb 4.6 ± 6.5%, P = 0.0004) were significant. ΔHct and ΔHb also correlated significantly with postnatal age (Fig. 2). Since baseline arterial lactate concentration and hematocrit varied significantly with postnatal age (r = −0.52, P = 0.0002 and r = −0.42, P = 0.003, respectively), percent changes may have been skewed proportional to the change in baseline value. However, analysis of absolute change in Hct and Hb with hypoxemia to postnatal age demonstrated significant relationships (ΔHct r = 0.41, P = 0.003; ΔHb r = 0.55, P = 0.001). Absolute change in lactate (ΔLact) to postnatal age was of borderline significance (r = 0.35, P = 0.018). Thus, increasing postnatal age was associated with greater hypoxemia-induced increases in arterial lactate, Hct and Hb, as well as osmolality.

Several renal function variables also responded to acute hypoxemia in a postnatal-age-dependent manner. Although the percent change of urinary

![Figure 3](a: The percent change in urinary flow rate in response to hypoxemia (ΔV) in relation to postnatal age; n = 37, r = 0.66, P = 0.0001. b: The percent change in urinary sodium excretion rate in response to hypoxemia (ΔU Na,V) in relation to postnatal age; n = 36, r = 0.65, P = 0.0001. c: The percent change in osmolar clearance rate in response to hypoxemia (ΔCosm) in relation to postnatal age; n = 34, r = 0.60, P = 0.0002. d: The percent change in glomerular filtration rate in response to hypoxemia (ΔGFR) in relation to postnatal age; n = 34, r = −0.01, P = 0.934.)
flow rate with hypoxemia was not significant for the group as a whole ($\Delta V - 10 \pm 77\%$, $P = 0.418$), $\Delta V$ correlated strongly with postnatal age (Fig. 3a). Similarly, the percent change of urinary sodium excretion rate with hypoxemia was not significant for the group as a whole ($\Delta U_{NaV} - 61 \pm 179\%$, $P = 0.048$), but was strongly correlated with postnatal age (Fig. 3b). Likewise, the percent change of osmolar clearance rate with hypoxemia was not significant for the group as a whole ($\Delta C_{\text{osm}} - 16 \pm 37\%$, $P = 0.015$), but correlated significantly with postnatal age (Fig. 3c). On the other hand, the percent change of fractional sodium excretion rate with hypoxemia ($\Delta F_{\text{Na}} - 120 \pm 240\%$, $P = 0.007$) had only borderline correlation with postnatal age ($r = 0.40$, $P = 0.019$). These significant relationships with postnatal age were paralleled by the percent change of urinary chloride excretion rate with hypoxemia ($r = 0.60$, $P = 0.0002$). Although glomerular filtration rate decreased significantly in response to hypoxemia ($\Delta GFR - 22.6 \pm 32.6\%$, $P = 0.0003$) in the group as a whole, the change in GFR in response to hypoxemia was not significantly correlated with postnatal age in these lambs (Fig. 3d). Only GFR among these variables had significant correlation of baseline values with postnatal age (GFR, expressed as ml/min, $r = 0.67$, $P = 0.0001$). However, absolute change in GFR with hypoxemia failed to correlate significantly with postnatal age ($\Delta GFR r = 0.36$, $P = 0.038$). Thus, increasing postnatal age was associated only with greater hypoxemia-induced declines in V and Cosm, and an increase in $U_{NaV}$.

Renal hemodynamic responses to acute hypoxemia in these lambs are illustrated in Figure 4. Mean arterial pressure did not change significantly in response to hypoxemia in the group as a whole ($\Delta MAP 3.4 \pm 15\%$, $P = 0.141$). However, baseline MAP ($r = 0.49$, $P = 0.0007$), $\Delta MAP$, expressed as absolute value ($r = 0.45$, $P = 0.0002$), and $\Delta MAP$, expressed as percent (Fig. 4a), were correlated significantly with postnatal age. Renal blood flow similarly did not decrease significantly in response to hypoxemia in the group as a whole ($\Delta RBF - 14.5 \pm 37.72\%$, $P = 0.22$). However, baseline RBF, expressed as ml/min, correlated significantly with postnatal age ($r = 0.60$, $P = 0.0001$), but was not significant when expressed as ml/min per g ($r = 0.38$, $P = 0.017$). Furthermore, RBF did not correlate with postnatal age when expressed as change in absolute value with hypoxemia ($r = 0.30$, $P = 0.061$) or when expressed as percent change (Fig. 4b). It is interesting to note that young lambs responded to acute hypoxemia with either an increase or decrease in RBF, but older lambs generally responded with only a decrease in RBF (Fig. 4b). Similar to RBF, correlation of baseline RVR to postnatal age was not significant ($r = -0.41$, $P = 0.017$) and the percent change in renal vascular resistance with hypoxemia ($\Delta RVR$) did not correlate significantly with postnatal age ($r = -0.29$, $P = 0.102$).

Responses to hypoxemia of selected hormones involved in renal function are depicted in Figure 5. Baseline values of these variables did not correlate significantly with postnatal age. Plasma renin activity increased significantly in response to hypoxemia, but the change in plasma renin activity with hypoxemia ($\Delta PRA$) did not correlate significantly with postnatal age ($r = 0.01$, $P = 0.99$). Similarly, plasma aldosterone concentration increased significantly in response to hypoxemia, but the change in plasma aldosterone concentration with hypoxemia ($\Delta Aldo$) did not correlate significantly with postnatal age ($r = 0.18$, $P = 0.230$). Likewise, plasma arginine vasopressin concentration increased in response to hypoxemia, but the change in plasma arginine vasopressin concentration with hypoxemia ($\Delta AVP$) did not correlate significantly with postnatal age ($r = 0.19$, $P = 0.199$). In contrast, urinary prostaglandin E excretion rate did not change significantly in response to hypoxemia, nor was the change in urinary prostaglandin E excretion rate with hypox-
study. Since AGFR was not significantly correlated increased change in urinary sodium chloride excretion with postnatal age, the greater saluretic response in older lambs was probably due to an influence acting directly on the renal tubules. Urinary excretion of a potential endogenous mediator of this saluresis, prostaglandin E (Dunn and Hood, 1977), which appears in urine at a rate correlating with its renal synthesis (Frölich et al., 1975), did not parallel the saluresis in response to hypoxemia. Similarly, serum aldosterone increased in a non-age-related fashion in response to hypoxemia. There is no evidence, therefore, that age-related changes in renal PGE or serum aldosterone mediated the age-related natriuretic effect of hypoxemia. Other potential mediators of these renal excretory responses, such as kinins and catecholamines, were not examined.

Postnatal age-related increases in blood lactate, hematocrit, and hemoglobin in response to hypoxemia in the present study may reflect greater changes in intravascular volume in older lambs under these conditions. This hypothesis is supported by studies in adult experimental animals demonstrating increased hematocrit (Jain et al., 1978) and decreased plasma volume (Hannon et al., 1969; Chinn and Hannon, 1970; Christensen et al., 1975; Jain et al., 1978) in response to acute high altitude hypoxemia. The rapidity of these changes in the present study and the rapid return to baseline values during recovery periods in other studies (Hannon et al., 1960; Chinn and Hannon, 1970) suggest that these changes may result from an acute net shift of fluid from the intravascular compartment during hypoxemia. Furthermore, the significant increase in serum osmolality in response to hypoxemia in the current study suggests that this fluid is free water which shifts from the intravascular to the intracellular compartments under these conditions, as suggested by Battaglia et al. (1958). The proximate cause of this fluid shift and the reason(s) for the significantly different response in younger lambs are not known. One might speculate that greater relative extracellular fluid volume in the younger lambs, as has been demonstrated in young humans (Friis-Hansen, 1961), may mask these fluid shifts.

In summary, the present study demonstrated in unanesthetized lambs that acute normocapnic hypoxemia induced: (1) significant percent increases in serum osmolality, blood lactate concentration, hematocrit, hemoglobin concentration, plasma renin activity, plasma aldosterone, plasma vasopressin and fractional sodium excretion rate; and (2) a significant percent decline in glomerular filtration rate. These results suggest that normocapnic hypoxemia has effects on the kidney which may contribute to asphyxial renal failure. Furthermore, several postnatal age related differences in the renal response to acute hypoxemia were noted, including (1) greater percent increases in serum osmolality, hematocrit, hemoglobin and lactate with increasing postnatal age, suggesting greater water movement from the intravascular fluid compartment during...
hypoxemia in more mature lambs; and (2) greater percent increases in urinary flow rate, urinary excretion rate of sodium chloride, and osmolar clearance rate with increasing postnatal age, suggesting greater effect of hypoxemia on the renal tubules of the older lambs to decrease sodium chloride reabsorption. These age-related effects do not support the hypothesis that the immature kidney is more susceptible to hypoxemia as a component of asphyxial renal failure.

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