Vasopressin Effects on Lung Liquid Volume in Fetal Sheep

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ABSTRACT

The normal switch from placental to pulmonary gas exchange at birth requires rapid removal of liquid from the lungs. Previous reports contend that vasopressin may be important in regulating this process, but this notion derives from studies in which fetal sheep received very large doses of vasopressin that yielded plasma concentrations at least 10 times greater than those that have been measured during normal labor. To study the physiologic effects of vasopressin on lung liquid volume in fetal sheep, we made three sets of experiments. First, we measured plasma vasopressin concentrations [VP] in 15 late-gestation fetal sheep, five of which were at various stages of spontaneous labor. [VP] in these fetuses ranged from <1 (prelabor) to 31 (during labor) $\mu\text{U/mL}$; postmortem extravascular lung water (EVLW) ranged from 4.5 to 14.5 g/g dry lung tissue. In a second series of studies, we measured EVLW in five sets of near-term (138 ± 1 d, term = 147 d) twin fetal sheep that received an 8-h i.v. infusion of either isotonic saline (control twin) or AVP (AVP-treated twin) at a rate of ~ 1 (mU/kg)/min. This dose was chosen to mimic [VP] measured in fetuses that had been studied during labor. [VP] did not change in the control twins, whereas [VP] increased from 1.8 ± 1.0 to 27.7 ± 3.5 $\mu\text{U/mL}$ in treated twins. There was a small, statistically significant difference in EVLW between twins that received AVP and untreated twins (11.9 ± 1.8 versus 14.6 ± 2.8 g/g dry lung). In a third series of studies, we measured

net production of lung liquid (Jv) by an indicator dilution technique in 16 fetal sheep that received an i.v. infusion of isotonic saline for 2 h followed by AVP for up to 8 h. [VP] averaged 23.4 ± 6.5 $\mu\text{U/mL}$ during AVP infusion. For six fetuses that were studied at <132 d (128 ± 4 d) gestation, Jv did not change during AVP infusion (11.1 ± 6.7 mL/h versus control, 10.2 ± 4.7 mL/h); for 10 fetuses >132 d (135 ± 2 d) gestation, Jv decreased from 11.3 ± 4.7 mL/h during the control period to 8.6 ± 5.4 mL/h during AVP infusion. Net secretion of lung liquid did not switch to net absorption in any of the 16 fetuses that received AVP. Thus, i.v. infusion of AVP, at a rate designed to mimic [VP] measured during spontaneous labor, led to modest reductions in Jv and EVLW, but did not cause net absorption of lung liquid in late-gestation fetal sheep. (*Pediatr Res* 38: 30–35, 1995)

Abbreviations

[VP], plasma concentration of vasopressin

EVLW, extravascular lung water

Pao₂, Pco₂ in arterial blood

Paco₂, Pco₂ in arterial blood

Jv, net production of lung liquid (rate of change in cumulative lung liquid volume over time)

During fetal life, the lungs are filled with liquid that forms as a result of epithelial Cl[−] secretion into the lumen of the developing respiratory tract (1). Rapid removal of this liquid from potential airspaces is an essential step in establishing an effective switch from placental to pulmonary gas exchange at

birth. There is abundant evidence that this process of liquid clearance normally begins before birth and is driven by active transepithelial sodium flux toward the lung interstitium (2, 3), with subsequent absorption of liquid into the pulmonary circulation and lymphatics (4). What regulates this process remains unclear, but several studies have suggested that a birth-related release of specific hormones, notably epinephrine (5) and vasopressin (6–8), into the fetal circulation may contribute to rapid absorption of liquid from the lungs before air-breathing begins.

Published reports concerning the effects of vasopressin on lung liquid removal describe studies conducted with fetal sheep

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and goats not during birth, but during i.v. infusion of AVP in the last month of gestation (6–11). Some studies have shown that the inhibitory effect of AVP on fetal lung liquid production is gestation-dependent, with little or no effect before ~130 d (term = 147 d) gestation (6, 7). With increasing fetal maturation, however, i.v. infusion of AVP consistently reduces net production of lung liquid and sometimes leads to liquid absorption late in gestation in the presence of labor. One study also showed a synergistic effect of epinephrine and AVP in reducing net production of lung liquid in fetal goats near term (6). In these studies, the inhibitory dose of AVP was sufficient to yield plasma concentrations of the hormone that were at least 10 times greater than those that have been measured in sheep during normal spontaneous labor and delivery (12, 13). Moreover, a recent report showed that AVP had no significant effect on the bioelectric properties of confluent monolayers of cultured lung epithelial cells harvested from late-gestation fetal rats (14). Thus, the importance of vasopressin in regulating lung liquid clearance near birth is uncertain.

The goal of this work was to determine the effects of i.v. infusion of AVP, at a dose designed to produce plasma concentrations approximating those that are measured during normal spontaneous labor, on the rate of production of lung liquid and on the amount of extravascular water in the lungs of fetal sheep late in gestation. We found that i.v. infusion of AVP had only a modest inhibitory effect on net production of lung liquid and on extravascular lung water content. These infusions did not induce net absorption of lung liquid in any of the sheep that we studied.

METHODS

Surgical preparation. By methods previously described, we surgically prepared 44 fetal lambs with chronic vascular catheters; 19 of these lambs also had a large-bore loop catheter that was inserted into the trachea for measurement of net lung liquid production, as described by Chapman *et al.* (15, 16). Time-dated pregnant ewes underwent surgery at 121–134 d of gestation. The sheep received ketamine, 500 mg intramuscularly, followed by either spinal anesthesia with 1% tetracaine, or general anesthesia with 1% halothane and nitrous oxide delivered with supplemental oxygen by a piston-type ventilator. We opened the uterus through a midline abdominal incision and exposed the fetal head and neck. If the ewe had received spinal anesthesia, we injected 1% lidocaine s.c. into the skin of the fetal neck, after which we inserted polyvinyl catheters into the carotid artery and jugular vein. In 19 fetuses, we incised the fetal trachea and inserted both ends of a saline-filled silicone rubber catheter (Silastic medical grade tubing, Dow Corning, Midland, MI; outside diameter 5.0 mm, inside diameter 2.6 mm, volume 17 mL). One end of this catheter was positioned just above the tracheal bifurcation and the other end just below the vocal cords, leaving a loop of catheter outside the fetus. We placed a smaller polyvinyl catheter (outside diameter 2.3 mm, inside diameter 1.3 mm) in the lumen of the distal trachea for subsequent pressure monitoring. The trachea was closed tightly around both catheters so that liquid flowed from the lungs through the catheter loop and

back into the upper airway. All fetuses had a catheter sutured to the outside of the neck, with the tip of the catheter in the amniotic sac, providing a zero reference for subsequent pressure measurements. Skin incisions were closed and all catheters were tunneled through the uterine and abdominal walls, which were doubly oversewn to prevent fluid leakage. A pouch was sewn to the maternal flank to protect the catheters from damage.

We injected antibiotics into the amniotic sac (10^6 U penicillin and 400 mg kanamycin) and fetal vein (300,000 U penicillin and 30 mg kanamycin) at the time of surgery and daily thereafter. Vascular catheters were flushed with isotonic saline and filled with heparin (1,000 U/mL) daily. All operative procedures and experimental protocols were approved by the Committee on Animal Use at the University of California San Francisco.

Experimental protocol. Sheep were allowed to recover from surgery for at least 3 d before experiments began. Studies were done on unanesthetized fetuses that were judged to be healthy by arterial pH and blood gas tensions. The fetuses were studied while their ewes stood upright in a cage with free access to food and water. In 15 late-gestation fetuses, we obtained samples of fetal blood for measurement of [VP]; five of these fetuses were in active labor that was detected by the presence of regular and repetitive increases in amniotic liquid pressure that varied from 5 to 20 mm Hg (0.7–2.7 kPa). Samples of plasma were obtained by centrifugation of blood specimens, after which they were frozen at -80°C . Ewes in labor were killed with an i.v. overdose of sodium pentobarbital, and the fetal lungs were immediately removed for measurement of EVLW as described below. In the two sets of vasopressin infusion studies, we measured fetal arterial pH, Pao_2 , and Paco_2 hourly with a calibrated blood gas/acid-base analyzer (model 158, Corning, Medfield, MA). We continuously measured arterial, venous, tracheal, and amniotic liquid pressures with calibrated transducers (Statham BT3DC and 131TC; Statham Instruments, Oxnard, CA) connected to an eight-channel amplifier-recorder (model 7D polygraph, Grass Instruments, Quincy, MA). Vascular and tracheal pressures were expressed relative to amniotic liquid pressure. All variables were measured during a 2-h baseline period (in which the vasopressin vehicle was infused) and throughout the i.v. vasopressin infusion, which lasted 2–8 h.

In studies designed to measure lung liquid production over time, samples of lung liquid were withdrawn at 10-min intervals to determine the rate of lung liquid production, as described below. We also did seven control experiments of lung liquid production for up to 10 h, in which the fetuses received no drugs.

Measurement of [VP]. We obtained samples of arterial blood in chilled, heparin-coated syringes, and the blood samples were immediately spun at 4°C . The plasma was withdrawn and stored at -80°C for 2–6 wk until extraction and assay by the method of Skowsy *et al.* (17). Briefly, vasopressin was extracted by absorption onto bentonite, followed by elution with acetone and separation with a 50:50 mixture of acetone:petroleum ether. The dried specimens were sealed and transferred to an independent laboratory (NASA-Ames Re-

search Center, Moffet Field, CA), where the samples were reconstituted and [VP] measured by RIA. The assay has a coefficient of variation of ~7% for intraassay measurements and ~15% for interassay measurements (17).

AVP infusion studies. Fetuses first received a 2-h i.v. infusion of vehicle at the beginning of each experiment (control period). The vehicle was a solution of chlorbutanol, 1 $\mu\text{L/mL}$ of isotonic saline, that was infused at a rate of 10 mL/h. This 2-h control period was followed by an i.v. infusion of AVP for 2–8 h. A stock solution (20 U/mL of isotonic solution with chlorbutanol, 1 $\mu\text{L/mL}$) of AVP (Quad, Indianapolis, IN) was diluted in isotonic saline to a concentration of 16–20 mU/mL for infusion into the fetal vein at a rate of 10 mL/h. This was calculated to achieve a vasopressin dose of ~1 (mU/kg)/min for an estimated fetal weight of 2.8–3.5 kg at 128–139 d of gestation. This dose was selected on the basis of our previous work in fetal lambs which indicated that a vasopressin infusion rate of 1 (mU/kg)/min would yield steady-state plasma concentrations of 15–30 $\mu\text{U/mL}$ (18), comparable to levels measured during spontaneous labor (12, 13).

Measurement of lung liquid production. We measured Jv by an indicator dilution method that was previously described by Walters and Olver (19) and modified by Chapman *et al.* (15, 16). At the start of each experiment, the tracheal loop was cut at its midpoint and lung liquid was withdrawn into a large, warm syringe to permit mixing and withdrawal of samples. We injected into the lung liquid a radiolabeled tracer (^{125}I human serum albumin, Mallinckrodt Inc., St. Louis, MO) that does not penetrate the epithelium, and we mixed the tracer in the liquid by gentle withdrawal and infusion several times over a 20-min period. Tracheal pressure did not exceed 20 cm H_2O (2.0 kPa) during this mixing procedure. We then removed 1–2 mL samples of lung liquid every 10 min for the duration of the experiment and measured the radioactivity (γ counter, Beckman Instruments, Palo Alto, CA) in duplicate 100- μL aliquots of each liquid sample. Gentle withdrawal and reinfusion of lung liquid was repeated between liquid samplings to assure uniform distribution of the albumin tracer within the lung lumen. The size of each liquid sample was adjusted to keep luminal liquid volume nearly constant. We obtained plasma samples periodically to assure that the albumin tracer remained within the lung lumen over the time course of these experiments; there was no detectable radioactivity in plasma. We made 23 experiments using 19 fetuses. Fetuses that were studied more than once had samples of lung liquid taken at the start of each subsequent experiment to measure background radioactivity in the lung liquid. After instillation of fresh radiolabeled tracer, sequential radioactive counts were adjusted by subtracting the background count for each sample.

Extravascular lung water measurements. At the end of some of the experiments, we inserted copper plugs into the vascular catheters and performed spinal anesthesia with 1% tetracaine on the ewe to deliver the fetus by cesarean section. We then injected a lethal i.v. dose of sodium pentobarbital, 25 mg/kg, rapidly split the sternum, occluded the trachea to prevent loss of lung liquid, aspirated blood from the heart, and clamped both hili. The lungs were resected, homogenized, and

the amount of extravascular water was determined by a gravimetric technique (20).

Data analysis. We calculated the volume of liquid within the lung lumen at the start of each experiment by determining the concentration of ^{125}I -albumin after thorough mixing. We then calculated the cumulative volume of lung liquid at each time point based on the dilution of ^{125}I -albumin, with appropriate corrections for the radioactivity and liquid volumes removed in previous samples. We calculated by least squares regression analysis the rate of change in cumulative lung liquid volume over time (Jv). Jv represents the sum of liquid secretion and absorption, processes that may coexist in different regions within the respiratory tract. A positive value for Jv indicates net production, a negative value represents net absorption.

Statistics. Results are expressed as mean values $\pm$ 1 SD. We used a paired *t* test to compare data obtained from fetuses before and during AVP infusion; we used an unpaired *t* test to compare data obtained from fetuses that received i.v. saline infusion *versus* those that received AVP, and to compare fetuses with and without labor. We defined as statistically significant those differences that were supported by a *p* value of <0.05 .

RESULTS

Studies of fetal lambs with and without labor. Arterial blood samples were obtained from 15 fetal lambs at 136–144 d of gestation. There were no significant differences between fetuses with or without labor with respect to arterial pH and blood gas tensions (data not shown). In the initial series of studies designed to determine [VP] in fetal sheep before and during labor, [VP] averaged $0.9 \pm 0.6 \mu\text{U/mL}$ (range: 0–2.8 $\mu\text{U/mL}$) and EVLW averaged $12.3 \pm 0.8 \text{ g/g dry lung}$ (range: 9.9–14.7 g/g dry lung excluding blood) for 10 fetuses that were killed without labor. In five fetuses that were killed during spontaneous labor, [VP] averaged $11.1 \pm 11.6 \mu\text{U/mL}$ (range: 3.4–31.0 $\mu\text{U/mL}$), and EVLW averaged $6.5 \pm 1.5 \text{ g/g dry lung}$ (range: 4.5–8.6 g/g dry lung).

Studies of EVLW in twin fetuses $\pm$ i.v. AVP infusion. Five pairs of twin lambs were studied at 138 ± 1 d of gestation. There were no significant differences between saline-treated and AVP-treated fetuses with respect to arterial pH and blood gas tensions (data not shown). Compared with saline-treated controls, AVP infusion caused significant increases of [VP] and arterial blood pressure, and a significant decrease in heart rate (Table 1). Body weight was similar for saline-treated and AVP-treated twins ($2.9 \pm 0.4 \text{ kg}$), and EVLW was significantly less in the fetuses that received AVP ($11.9 \pm 1.8 \text{ versus } 14.6 \pm 2.8 \text{ g/g dry lung tissue}$ for control fetuses).

Studies of net lung liquid production. In seven control experiments performed with fetuses (131 ± 3 d of gestation) that received i.v. saline for up to 10 h, there was no change in Jv between the first and last 2 h of study, nor were there significant changes in any of the measured variables over time in these seven studies (Table 2).

The i.v. infusion of AVP did not alter Jv in fetuses <132 d of gestation, but Jv decreased on average by 24% during AVP infusion in fetuses >132 d of gestation (Table 2). For the six

Table 1. Data from studies in which five sets of twin fetal sheep (gestation 138 ± 1 d) received i.v. infusions of either isotonic saline or AVP for 8 h after a 2 h control period

Fetuses	Period	Body weight (kg)	Mean arterial blood pressure (kPa)	Heart rate (beats/min)	[VP] (μ U/ml)	EVLW (g/g dry lung)
Control	Baseline	2.75 ± 0.36	7.5 ± 0.8	171 ± 23	1.9 ± 0.6	14.6 ± 2.8
	Saline infusion		7.2 ± 0.5	170 ± 25	2.6 ± 1.5	
AVP	Baseline	2.87 ± 0.45	7.1 ± 0.3	165 ± 11	1.6 ± 0.9	$11.9 \pm 1.8^\dagger$
	AVP infusion		$8.0 \pm 0.8^{*\dagger}$	$145 \pm 16^{*\dagger}$	$27.3 \pm 7.4^{*\dagger}$	

Values are mean $\pm$ SD; to convert kPa to mm Hg, multiply by 7.5.

* Significant difference compared to baseline period, $p < 0.05$.

$\dagger$ Significant difference compared to fetuses that received i.v. saline, $p < 0.05$.

Table 2. Data from studies in which Jv was measured in fetal lambs that received i.v. infusion of either vehicle (control) or AVP

Fetuses	Number	Period	Gestation (d)	Initial lung liquid volume (ml)	Mean arterial blood pressure (kPa)	Heart rate (beats/min)	[VP] ‡ (μ U/ml)	Jv (ml/min)
Control	7	Baseline	131 ± 3	98 ± 23	7.0 ± 0.9	173 ± 27		13.0 ± 2.4
		Vehicle infusion			7.0 ± 0.9	173 ± 22		13.0 ± 2.9
AVP (<132 d)	6	Baseline	128 ± 2	102 ± 25	6.4 ± 1.1	177 ± 12	6.1 ± 5.0	10.2 ± 4.7
		AVP infusion			$7.5 \pm 1.1^*$	$159 \pm 11^*$	$25.8 \pm 4.2^*$	11.1 ± 6.7
AVP (>132 d)	10	Baseline	135 ± 2	107 ± 26	6.7 ± 0.8	169 ± 20	6.0 ± 4.4	11.3 ± 4.7
		AVP infusion			$7.5 \pm 1.1^*$	$138 \pm 23^{*\dagger}$	$22.4 \pm 7.4^*$	$8.6 \pm 5.4^*$

Values are mean $\pm$ SD; to convert kPa to mm Hg pressure, multiply by 7.5.

* Significant difference compared to baseline period, $p < 0.05$.

$\dagger$ Significant difference compared to control fetuses, $p < 0.05$.

$\ddagger$ [VP] measured in three fetuses <132 d and seven fetuses >132 d of gestation.

fetuses that were studied at a gestational age of <132 d (128 ± 2 d), Jv averaged 10.2 ± 4.7 mL/h during the control period and 11.1 ± 6.7 mL/h during AVP infusion. For the 10 fetuses that were studied at a gestational age of >132 d (135 ± 2 d), Jv averaged 11.3 ± 4.7 mL/h during the control period and 8.6 ± 5.4 mL/h during AVP infusion.

Mean arterial blood pressure increased (6.6 ± 0.8 to 7.5 ± 1.1 kPa) and heart rate decreased (172 ± 17 to 145 ± 22 beats/min) significantly during AVP infusion. These changes were consistent irrespective of gestational age. There were no significant changes in arterial pH or blood gas tensions associated with AVP infusion (data not shown). We obtained paired arterial blood samples before and during AVP infusion in 10 of the 16 experiments: [VP] averaged 6.0 ± 4.3 μ U/mL during the control period and 23.4 ± 6.5 μ U/mL during AVP infusion.

DISCUSSION

The importance of a hormone in regulating a body function depends on its action at normal physiologic concentrations. The results of our studies indicate that i.v. infusion of AVP, at a rate designed to mimic plasma concentrations measured during normal labor, causes small but significant decreases in both lung liquid production and extravascular lung water in late-gestation fetal sheep. Net absorption of lung liquid did not occur in any of our vasopressin infusion experiments. However, we cannot exclude the possibility that vasopressin might have a role in removing liquid from the lungs during normal spontaneous labor at term gestation, where the role of epinephrine remains controversial (4, 5, 16, 21).

Several reports have shown that umbilical cord [VP] increases at birth both in human infants (22–27) and newborn

sheep (12, 13, 28). Values are greatest after spontaneous vaginal delivery. Previous studies showed that [VP] in late-gestation fetal sheep averaged <10 μ U/mL and increased up to 5-fold during spontaneous labor, with plasma concentrations in umbilical cord blood averaging 10–30 μ U/mL (12, 13, 28).

These observations provided the rationale for several groups of investigators to test the effects of AVP on lung liquid production in fetal goats and sheep (6–11). Perks and Cassin (9) infused AVP i.v. into fetal goats at a rate of 1.6–39.2 (mU/kg)/min for 1 or 2 h. Five fetuses <127 d of gestation showed no response in lung liquid production, whereas 16 fetuses >131 d of gestation had progressive reduction in lung liquid secretion, with net liquid absorption in some of the fetuses. These were acute studies conducted in the presence of anesthesia immediately after surgery, conditions that may stimulate endogenous release of vasopressin (12, 22) and other stress-related hormones, including catecholamines. These authors estimated a threshold effect on lung liquid production that became evident at an infusion rate of ~ 1.3 (mU/kg)/min, similar to the rate that we used in our studies. The same investigators also studied eight chronically catheterized fetal sheep and found that i.v. infusion of AVP decreased lung liquid secretion in four fetuses >134 d of gestation (29). In these studies, however, the rate of infusion of AVP was 7.5–20.5 (mU/kg)/min, several times greater than the rate of infusion that we used. These investigators found that the effect of AVP infusion on lung liquid production was both dose- and gestation-dependent (6, 29).

In their 1979 report on [VP] in fetal sheep before and during spontaneous vaginal delivery, Stark *et al.* (13) found that [VP] averaged 2 ± 3 pg/mL (1 pg = 0.36 μ U; AVP units may vary according to the specific preparation that is used) on the day

before delivery, whereas [VP] measured during labor in four fetuses averaged ~ 21 pg/mL, increasing to 26 pg/mL in umbilical cord blood. This report included the results from one additional fetal sheep, in which [VP] was 4,667 pg/mL during labor and 8,000 pg/mL in umbilical cord blood. This animal had severe respiratory distress from birth and died 3 h after delivery. The authors noted that [VP] measured in this preterm lamb was comparable to plasma concentrations measured in portal hypophyseal blood. This animal was atypical on several counts: it was premature, measurements of [VP] before labor were not recorded, death occurred within 3 h of birth, and [VP] measured during labor and in cord blood were clearly aberrant from other published values during labor. Yet the data reported for this single animal subsequently served as rationale for infusing large doses of vasopressin into otherwise healthy fetal sheep to determine their "physiologic response" in terms of lung liquid production. For example, Wallace *et al.* (7) infused AVP at a dose of 300 (mU/kg)/h for 2 h and reported plasma concentrations that averaged 849 ± 75 pg/mL, a dose that was sufficient to induce lung liquid absorption in fetal sheep close to term gestation. Cassin and Perks (8) infused AVP at a rate that averaged almost 1500 (mU/kg)/h for 7 h, and although they did not measure plasma concentrations in these fetal sheep, it is likely that they were at least as great as the plasma concentrations measured by Wallace *et al.* (7). In these studies, prolonged infusion of AVP at this very high dose substantially inhibited lung liquid production and in some cases led to liquid absorption; intrapulmonary administration of amiloride, an inhibitor of conductive sodium transport, reversed this effect (8, 11). Because plasma concentrations in these animals were at least 10 times greater than those measured during normal labor in sheep, the physiologic significance of these studies remains uncertain.

Are there other data to support the use of such high doses of AVP in these earlier studies? A number of investigators have shown that plasma concentrations of vasopressin may increase significantly in fetal sheep that undergo the stress of severe hypoxemia (30, 31), hemorrhage (32), or premature labor induced by i.v. infusion of ACTH (33). Moreover, [VP] measurements in umbilical cord blood of vaginally delivered human infants are considerably greater than they are in fetal sheep (22–26, 34, 35). Values are greater in umbilical arterial than in umbilical venous blood, with plasma concentrations sometimes in excess of 600 μ U/mL (25, 34, 35).

In our studies, we chose an i.v. infusion rate of AVP that would increase plasma concentrations to 20–30 μ U/mL, comparable to those that we and others have measured during labor. These infusions caused only modest decreases in lung liquid production and extravascular lung water of fetal sheep late in gestation. It is possible, however, that plasma concentrations of vasopressin measured in fetal sheep during spontaneous labor might contribute to clearance of lung liquid if there is an increase in the sensitivity of the epithelium to vasopressin at this later stage of lung development. This notion is analogous to the increased lung epithelial sensitivity to epinephrine that may develop in sheep during labor, wherein sodium and liquid absorption may occur even at plasma concentrations of epinephrine that are within the normal prelabor range (5). In

addition, the higher [VP] measured in umbilical cord blood of human infants raises the possibility that the aforementioned studies of high dose AVP infusions in fetal sheep might be relevant to lung liquid changes near birth in human infants, provided the human respiratory epithelium is sensitive to vasopressin.

The mechanism by which vasopressin decreases net production of luminal liquid in the fetal lung remains unclear. The i.v. infusion of AVP increases systemic and pulmonary arterial pressures (30, 36), which in turn could influence vascular filtration pressure. It is unlikely, however, that this hemodynamic change would affect liquid secretion by the respiratory epithelium, as we and others have shown that this active secretory process is unresponsive to modest changes in vascular filtration pressure (1, 37). The observation that intrapulmonary administration of amiloride reversed the inhibitory effect of AVP on lung liquid production led Cassin and Perks (8) to speculate that AVP may act by stimulating V_2 receptors in lung epithelium, with a resultant increase in adenylate cyclase and protein kinase A activity, culminating in activation of sodium channels on the luminal surface of the epithelium. Increased sodium uptake would be expected to stimulate Na^+/K^+ -ATPase on the abluminal surface of the epithelium, thereby driving liquid from the airspaces into the interstitium. This proposed mechanism is consistent with the observation by Goodman *et al.* (38) that AVP increased dome formation in confluent monolayers of cultured lung epithelial cells from adult rats. Contrary to this view, however, three other groups of investigators found no effect of AVP on the bioelectric properties of respiratory epithelium from fetal and adult rats (14, 39) and from mature rabbits (40). These observations support the skepticism expressed in a recent commentary on the possible role of vasopressin in stimulating lung liquid clearance by a sodium-dependent process at birth (41).

It is possible that i.v. infusion of AVP may cause release of epinephrine, which is thought to stimulate sodium uptake and liquid absorption by the pulmonary epithelium during and after birth (3, 5). This notion is certainly plausible in the setting of prolonged i.v. infusion of AVP at very high doses that are sufficient to induce stress. Nor can we exclude the possibility that AVP acts primarily on transmembrane water channels, rather than sodium channels, of respiratory epithelium, similar to the mechanism that has been described for AVP's action on other epithelia (42). Thus, the specific role of vasopressin in lung liquid clearance at birth is ill-defined and warrants further inquiry.

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