β-Adrenergic receptors lung
epinephrine   newborn
fetus   propranolol
isoproterenol

The Role of Catecholamines in Lung Liquid Absorption at Birth

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Summary

We have examined the effect on lung liquid secretion of catecholamines infused in chronically catheterized fetal lambs *in utero*. Isoproterenol and epinephrine inhibited secretion, an effect which increased with gestation and, in fetuses near delivery, caused absorption of lung liquid. In 7 out of 8 experiments nor-epinephrine had no effect on secretion. This pattern of response and the fact that the inhibitory effect could be blocked by propranolol indicate a mode of action involving β-adrenergic receptors.

Speculation

The observed effects on lung liquid secretion were achieved with infusion rates of epinephrine which are well within the range for endogenous production by the adrenals in response to splanchnic nerve stimulation or asphyxia. Thus the marked increase in the secretion of epinephrine which occurs during parturition may be an important factor in initiating lung liquid absorption. Permanent inhibition of lung liquid secretion postnatally may be due to greater sensitivity of the pulmonary epithelium to β-adrenergic stimulation and to the higher "resting" output of epinephrine from the adrenals of neonates compared with fetuses.

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Introduction

The lungs of the fetus are filled with a specialised secretion elaborated by the respiratory epithelium - a process which involves active ion transport (15). In the mature fetal lamb, lung liquid is formed at a rate of some 2-3 ml/hr.Kg body weight and occupies a volume roughly equivalent to the Functional Residual Capacity of the newborn: 30 ml/kg body weight (14). In the course of adaptation to air breathing, the liquid within the lung must be cleared to make way for air. Although it is well established that this process takes some 4-6 hours for completion (2, 11), how it is achieved is far from clear. Olver and Strang (15) showed that when active transport in the lung is inhibited by cyanide, absorption of lung liquid takes place down the protein osmotic gradient which exists between this fluid and plasma (- about 20 mmHg). However, the physiological mechanism for "switching off" lung liquid secretion at birth is not known. Certainly the secretory mechanism must be modified at this time, so that liquid does not subsequently accumulate in the lungs under normal conditions.

The amounts of catecholamines released in response to splanchnic nerve stimulation and asphyxia increase during gestation in the lamb (5, 7) and there is good circumstantial evidence for enhanced catecholamine secretion during parturition (6, 12). Furthermore, the output of epinephrine from "resting" (i.e. denervated) adrenals is greater in the newborn than in the fetus. For these reasons we chose to examine the effect on lung liquid secretion of certain catecholamines in chronic fetal lamb preparations in utero.

Methods

We performed experiments on lamb fetuses in nine chronically catheterized preparations. The animals used were Dorset Short Horn or Clun Forest ewes whose tupping dates were known.

Surgical Procedures:

Pregnant ewes between 115 and 125 days' gestation were anesthetized with intravenous pentobarbitone sodium ('Sagatal', May and Baker) 300-600 mg, and anesthesia maintained with repeated small doses. Under sterile conditions the uterus was approached through a midline abdominal incision. A three to four inch cut was made in the uterus near a fetal head, care being taken to minimise amniotic fluid loss. Two large bore catheters were tied into each end of the incised fetal trachea, and smaller catheters were inserted into a carotid artery and a jugular vein. The uterus and abdomen were closed in layers and the catheters brought out through the uterine incision and then through a flank incision some distance from the abdominal wound. A 5-day postoperative course of penicillin and streptomycin was routinely given to ewe and fetus.

Catheters:

All catheters were made of Silastic (Dow-Corning). The tracheal catheters had an internal diameter 0·264 cm and were cut to 100 cm length, giving a volume of 5·5 ml each. Between periods of experimental observations, the two catheters were joined to form a loop so that lung liquid could flow normally through the larynx. The pressure drop along the whole length of the tracheal loop was 1·0 mm of water at flow rates of 15 ml/hour (usual lung liquid secretion rates in our experiments were between 10 and 15 ml/hour). Blood catheters had an internal diameter of 0·102 cm and were cut to 150 cm length. Catheter volumes were 1·2 ml each and when not being used were filled with a heparin solution (500 units/ml).

The join in the tracheal loop and the junctions of the blood catheters with syringes were wrapped in spirit swabs and kept in sterile polythene bags on the back of the ewe.

The positions of the catheters after death were ascertained in 8 of the 9 lambs. The tips of the lung tracheal catheters usually produced a slight roughening of the tracheal epithelium but no other signs of damage were seen. The tips of the venous catheters lay between the lower end of the external jugular vein and the superior vena cava. The tips of the arterial catheters lay between the carotid artery and the arch of the aorta.

Experimental Procedure:

The first experiment on each fetus was performed not earlier than 6 days after the operation. Subsequent experiments were no more frequent than on alternate days.

The tracheal loop was broken at the join and a syringe attached to the catheter connected to the fetal lung. Lung liquid secretion rate was measured by the impermeant tracer technique (14). A known amount of radio-iodine (^{125}I or ^{131}I) labelled albumen was introduced into the lung liquid, which was then thoroughly mixed over a period of 30 minutes by repeated withdrawal and reinjection via the tracheal cannula. The total lung liquid volume at any time could be calculated from the tracer concentration and to determine this, lung liquid samples were taken every 5 or 10 minutes (a correction was applied for the small amount of tracer removed with each sample). We adjusted sample volume so that the volume remaining in the lung was approximately constant. After a control period of one hour, one of three catecholamines was infused via the venous catheter, usually for 70 minutes. The

ranges of infusion rates used were: isoproterenol 0·15 - 1·0 µg base/min, epinephrine 0·16 - 3·0 µg base/min and norepinephrine 0·18 - 3·0 µg/base min. In spite of prior filling of the catheter dead space with catecholamine solution, there was often a delay of about 10 minutes before there was any effect on lung liquid secretion. Therefore, secretion rates were calculated from 15 minutes after the start of the infusion. A 30-minute period of recovery was allowed after the end of the infusion and sampling was then continued for a further hour as a second control period. This sequence could be repeated with variations such as a different dose, or a different agonist or using an antagonist.

Monitoring of Blood Gases, Blood Pressure and Heart Rate:

Frequent measurements of pH, $PaCO_2$ and PaO_2 were made. During the control periods the mean value of pH was 7·423 (S.E.M. $\pm$ 0·013) of $PaCO_2$ was 44·4 ($\pm$ 1·2) mmHg and for PaO_2 25·8 ($\pm$ 1·0) mmHg. Infusion of the catecholamines did not produce a significant change in any of these values.

Fetal arterial blood pressure and heart rate were continuously monitored via the arterial catheter. The mean control heart rate was 160 ($\pm$ 6·4) beats/min. Isoproterenol produced a mean increase of 103 ($\pm$ 5·7) beats/min. (P <0·001) but had no significant effect on mean blood pressure. Epinephrine infusion caused an increase of 24 ($\pm$ 3·6) beats/min in heart rate (P <0·01) but had no effect on mean blood pressure. Norepinephrine infusion had no significant effect on heart rate, but produced an average increase of 6 ($\pm$ 2·0) mmHg in mean blood pressure (p <0·05).

Drugs and Chemicals:

Radiolabelled Albumen, The Radiochemical Centre Ltd., Amersham, Bucks, U.K.
Propranolol hydrochloride, "Inderal", I.C.I., Gt. Britain.
Norepinephrine 1:000, 'Levophed', Winthrop, England.
Epinephrine 1:1000, Antigen Ltd., Ireland.
Isoproterenol hydrochloride 1:5000, "Isuprel", Winthrop, England.

Solutions of catecholamines were made up immediately before each infusion in physiological saline, in such a strength that the volume infusion rate was always the same, viz. 8·8 ml/hour.

Analysis of Samples:

Weighed aliquots (about 0·4g) of the lung liquid samples were counted on a Packard Autogamma scintillation spectrometer with energy gates set between 10-80 KeV for ^{125}I and 100-450 KeV for ^{131}I. The counts of the latter isotope were decay corrected.

Acute Experiment:

On one of the ewes carrying twins we performed an acute experiment at 136 days' gestation, 20 days after the operation to set up the chronic preparation. The ewe was anesthetized with intravenous pentobarbitone and the twin which had not been previously operated on was delivered onto a warmed table, the umbilical circulation being left intact. Measurements of lung liquid secretion rate and infusion of isoproterenol were carried out as described for the chronic preparations.

Spontaneous Labour:

In the four sheep in which the effects of maturation on the response to infused isoproterenol were examined, pregnancy was allowed to proceed until the onset of spontaneous labour. This occurred at a mean gestational age of 135 days (range 132-137 days), whereas term in the sheep is usually considered to be 147 days. We attributed this shortening of gestation to the effects of surgery. The mean weight of the lamb fetuses at death was 3·53 ($\pm$ 0·29) kg.

Results

Beta-adrenergic effect:

Isoproterenol and epinephrine inhibited lung liquid secretion rate or caused absorption of lung liquid in 33 of 36 experiments. Nor-epinephrine had no effect in 7 out of 8 experiments. A summary of all these results is given in Table I.

An example of all the effects of the 3 catecholamines is given in Figure 1. Each point represents a volume for accumulated lung liquid (including that removed in samples) at time t calculated from the measured concentration of labelled albumen (C_t). At time t,

$$V_t = [(X - R) / C_t] + V_s$$

Where V_t is accumulated liquid volume, X is the amount of impermeant tracer initially added, R is the sum of the amounts of tracer removed in previous samples and V_s the sum of the volumes of all the previous samples. Calculated regression lines are shown; the slopes give the rate of secretion or absorption of lung liquid.

In this example, 5 minute samples were taken and the first slope gives the control secretion rate; its numerical value is shown just above the horizontal axis. Epinephrine was then infused; this caused lung liquid absorption as indicated by the negative

slope of the second regression line. After a thirty minute recovery period, the slope became positive again indicating that lung liquid was being secreted. Although the secretion rate had not returned to its initial value, infusion of nor-epinephrine had no effect; the slopes of the 3rd, 4th and 5th regression lines (before, during and after nor-epinephrine) were not significantly different (P > 0·30). However, during isoproterenol infusion absorption took place so rapidly that, after 30 minutes, insufficient liquid was present for further samples to be taken. It should be appreciated that although Figure 1 shows progressively increasing accumulated lung liquid volume, the volume inside the lung remained more or less constant at the beginning of each experimental period due to removal of samples). After 90 minutes, liquid had reaccumulated sufficiently in the lung to allow further sampling, and the secretion rate had returned to its initial value. The slopes of the first and last regression lines do not differ (P > 0·40). Most of the experiments comprised only one or two infusions of catecholamines.

The beta-adrenergic mode of stimulation suggested by these results was confirmed by the finding that the effects of isoproterenol and epinephrine could be blocked by propranolol (range 2.0 - 4·8 mg total, n = 5). The appropriate rate of propranolol infusion was determined empirically. In all cases the amount given was sufficient to block the cardiovascular response to the infused catecholamine but in two experiments it failed to block the inhibitory effect on lung liquid secretion. These observations suggest that the affinity of the cardiac receptors for propranolol may be appreciably greater than those of the lung epithelium; a point of some relevance to the clinical situation in which babies are born with their cardiovascular reflexes blocked following administration of propranolol to the mother. An example demonstrating blocking of the effect of isoproterenol on lung liquid secretion is shown in Figure 2. Other experiments showed that propranolol given alone had no effect on lung liquid secretion.

Maturation effect:

The effect of infused isoproterenol on lung liquid secretion increased with gestational age. In four sheep the dose of isoproterenol given early in gestation was repeated at least once at a later date. Figure 3 shows the results from a single fetus into which the same dose of isoproterenol was infused 4 times between 122 and 133 days' gestation. The response increased each time, absorption first occurring at 129 days' gestation and an even more rapid absorption at 133 days' gestation. Results from the four sheep are summarised in Table II. In this table it can be seen that the response to isoproterenol infusion (ΔJ_v) always increased with advancing gestation. This increase in ΔJ_v is not linear and accelerates towards term, i.e. there appears to be an exponential relationship between ΔJ_v and gestation. The slopes of the log-linear regression lines for ΔJ_v on gestation for the first three sheep are not significantly different from each other; therefore, we have pooled the data from these animals and added the two points from the fourth sheep. These results are shown graphically in Figure 4, which demonstrates a significant increase in ΔJ_v with increasing gestation (P < 0·001).

Acute experiment:

In the acute experiment performed at 136 days' gestation on a fetus which had not previously been operated on, but which was a twin of a fetus used for chronic experimentation, the infusion of 0·76 μg base/min of isoproterenol resulted in lung liquid absorption at 27 ml/hour after a control period during which the secretion rate was 9·9 ml/hour. This result renders unlikely the proposition that previous surgery or repeated doses of catecholamines were responsible for the results described above.

Discussion

These results show clearly that infused catecholamines inhibit the secretion of lung liquid and, particularly in the mature fetus, initiate its absorption. In achieving this effect both isoproterenol and epinephrine are much more potent than nor-epinephrine when comparison is made on an equimolar basis (Table I, Figure 1). This finding suggests that the effect on lung liquid secretion is mediated via beta-adrenergic receptors, an hypothesis borne out by the observation that inhibition of secretion can be blocked by propranolol (Figure 2). As shown in Table II and Figure 3, the sensitivity of the system increases with gestation and is maximal at term, at which time we have observed absorption, in response to infused beta-agonists, at rates of up to 40 ml/hr. This absorption of lung liquid has been achieved with epinephrine infusion rates which are well within the range for endogenous epinephrine secretion in response to splanchnic nerve stimulation or asphyxia in fetal lambs, and are of the same order of magnitude as the "resting" adrenal secretion rates of the newborn lamb (5, 7). Furthermore, we have observed absorption of lung liquid in the mature fetus in response to the infusion of epinephrine at rates appreciably less than that found to give plasma concentrations of epinephrine (1·0 - 3·6 ng/ml) comparable to levels following moderate hypoxia (12). Similar concentrations (mean: 2 ng/ml) have been measured in human umbilical artery blood after uneventful vaginal delivery, while asphyxia during delivery has been found to give much higher levels (13). Thus, it is quite possible that epinephrine has a causal role in lung liquid absorption at birth, and that this process may begin with the onset of labour. On the one occasion

on which we have made measurements during early labour, lung liquid was found to be undergoing spontaneous absorption at 6·0 mls/hr.

With the onset of ventilation, certain changes take place in the lung which can be expected to speed the clearance of liquid. Hydraulic conductivity of the epithelium increases as a result of the "pore stretching" phenomenon described by Egan et al (9), at a time when an hydrostatic gradient is established between airway and interstitium. Simultaneously pulmonary vascular resistance falls with a concomitant increase in lung blood flow. We have no information as to whether pulmonary blood flow changes during periods of catecholamine infusion in our experiments, but we believe that such changes cannot account for our findings - for while isoproterenol and epinephrine both inhibit lung liquid secretion, their reported effects on pulmonary vascular resistance in the lamb fetus are opposite (4, 8). Furthermore, Olver and Strang (15) have shown that alterations in pulmonary haemodynamics, sufficient to cause marked changes in pulmonary lymph flow, have no effect on the rate of lung liquid secretion. We therefore conclude that beta-adrenergic agonists have a specific and direct effect on the mechanism of lung liquid secretion at the level of the respiratory epithelium. Since the process of lung liquid formation involves active ion transport, the flow of solute and water being linked osmotically, inhibition of active solute movement would be expected to halt secretion. In the absence of any active mechanism, water will flow in response to whatever pressure gradients are present. In the fetal lung, plasma and interstitial protein osmotic pressure are some 20 mmHg higher than in lung liquid (3), and thus the direction of passive net volume flow will be from the potential air spaces into the circulation. Such a reversal of flow has been observed after poisoning of the chloride ion pump of the fetal respiratory epithelium with cyanide (15). However, we cannot exclude the possibility that the stimulation of adrenergic receptors may reverse the direction of net active ion transport and hence water flow. Such a phenomenon occurs in the small intestine in response to a variety of agents (10).

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Table I. Effect of catecholamines on foetal lung liquid secretion.

DRUG	DOSE Mean & Range (µg base/min)	NO. OF EXPERI- MENTS	GESTATION Range (Days)	EFFECT ON LUNG LIQUID SECRETION (No. of Experiments)		
				Absorption+	Inhibition++	None
Isoproterenol	0·69 0·15-1·0	21 (8 sheep)	121-136	8	11	2*
Epinephrine	0·60 0·16-3·0	15 (7 sheep)	118-133	2	12	1**
Nor-Epine- phrine	0·85 0·18-3·0	8 (5 sheep)	120-133	0	1***	7

Mean doses are in molar ratio of Isopr 1·0: Epi 1·0: Nor-Epi 1·5.
+ Absorption = inhibition of secretion with reversal of direction of flow.
++ Inhibition = significant decrease in flow (p < 0·05) without absorption.
* Both at 122 days' gestation. ** At 123 days' gestation. *** 3 µg/min at 130
  days.

Table II. Effect of maturation on response to isoproterenol.

SHEEP NO.	ONSET OF LABOUR * (Days)	GESTATION (Days)	LUNG LIQUID SECRETION RATE, J_v (ml/hr)		ΔJ_v (ml/hr)
			Control	Isoproterenol	
1303	137	124	10·1	7·7	6·4
		127	11·2	2·8	8·4
		131	12·8	4·3	8·5
		134	6·9	-18·8	25·7
1309	136	122	13·2	11·1	2·1
		126	19·1	4·5	14·6
		129	8·9	- 9·0	17·9
		133	10·5	-41·5	52·0
1313	133	126	13·5	6·5	7·0
		129	16·2	7·2	9·0
		131	13·1	0·3	12·8
		133	13·0	-11·9	24·9
1314	132	127	11·3	- 1·8	13·1
		130	15·3	-14·9	30·2

Minus sign denotes absorption.
* Gestational age at onset of labour.
ΔJ_v is control secretion rate minus secretion rate during
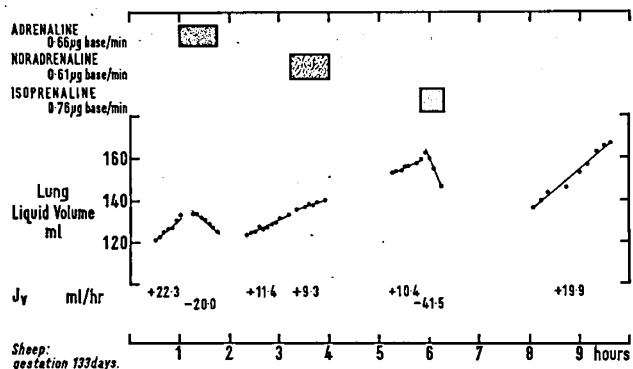isoproterenol infusion.

Figure 1. Effect of intravenous catecholamines on fetal lung
liquid secretion. Values of accumulated lung liquid volume were
calculated as described in the text. The change in lung liquid
volume with time (J_v) in any period was obtained from the regres-
sion line fitted to the points. Positive values for J_v indicate
secretion, negative values absorption. Epinephrine (Adrenaline),
Nor-epinephrine (Nor-adrenaline) and Isoproterenol (Isoprenaline)
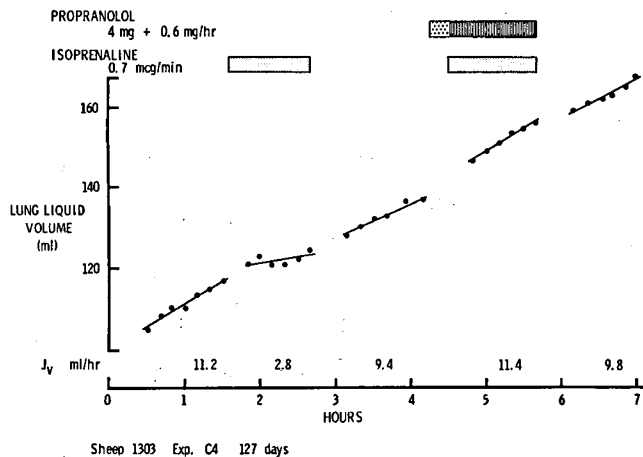were infused at equimolar rates.

Figure 2. Blocking of the effect of isoproterenol (Isoprenaline)
on fetal lung liquid secretion by propranolol. Both drugs were
given intravenously. The infusion of propranolol was preceded by
a loading dose of 4 mg of propranolol given over 15 minutes.
Isoproterenol alone caused inhibition of secretion; the slope of
the second regression line differs significantly from those of
the controls on each side (P < 0·01). The slope of the fourth
regression line (during Isoproterenol + Propranolol infusion) is
not different from the controls on each side (P > 0·1). The
numerical value of each slope gives the secretion rate (J_v) during
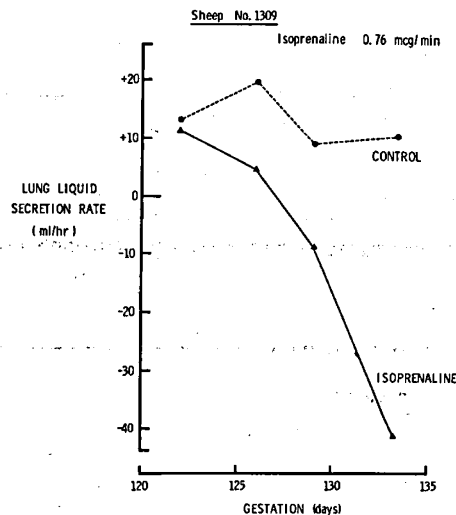that period.

Figure 3. Increasing effect of Isoproterenol (Isoprenaline) on
lung liquid secretion with advancing gestational age in a single
fetus. The same dose of Isoproterenol (0·76 µg/min) was infused
at 4 gestational ages. Negative values for lung liquid secretion
rate indicate absorption. ● = control lung liquid secretion and
▲ = the secretion (or absorption) rate during Isoproterenol
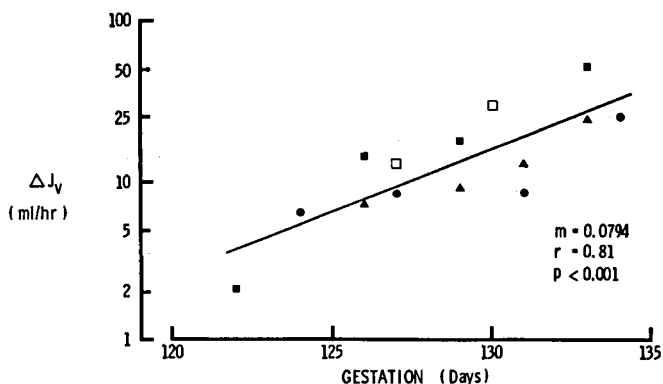infusion which immediately followed the control period.

Figure 4. Increasing effect of Isoproterenol (Isoprenaline) on
fetal lung liquid secretion with advancing gestational age -
pooled data from Table 2. ΔJ_v plotted logarithmically.
(ΔJ_v = the difference between control secretion rate and that
observed during Isoproterenol infusion). ● values from sheep No.
1303, ■ from sheep No. 1309, ▲ from sheep No. 1313 and □
from sheep No. 1314.