

THE EFFECTS OF ALPHA ADRENERGIC RECEPTOR STIMULATION,
USING EPINEPHRINE, ON LUNG FLUID PRODUCTION IN *IN VITRO*
PREPARATIONS OF LUNG FROM FETAL GUINEA PIGS
(*Cavia porcellus*).

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ABSTRACT

This study examined the effects of epinephrine on fluid production and reabsorption by fetal guinea pig lungs. Catecholamine levels in the fetus have been found to increase significantly near term and epinephrine has been proposed to play a role in the reabsorption of lung fluid. Improper fluid reabsorption at birth leads to a condition called respiratory distress syndrome which is the leading cause of death among newborns.

Our procedure involved supporting the lungs in vitro for a duration of three hours in Krebs' saline and the rates of fluid production were measured using a dye-dilution technique. The control rates ranged from 0.98 to 1.34 ml/kg·hr with gestational ages between 59 to 65 days (gestation is 67 days) which is comparable to rates reported from fetal sheep.

The lungs were exposed to different concentrations of epinephrine, ranging from 10^{-8} M to 10^{-5} M, during the second hour of experimentation, following an ABA design. Epinephrine produced reductions in fluid secretion in a dose dependent manner except at pharmacological levels where the response disappeared. Optimal responses to epinephrine were obtained at 10^{-7} M. The unexpected results at high concentrations of epinephrine warranted further investigation using specific alpha-adrenergic receptor blockers.

However, tests with general alpha and beta-adrenergic receptor antagonists were carried out beforehand to confirm previous work which showed that epinephrine works through alpha and not beta receptors. Propranolol, a general beta blocker, did not block the effect of 10^{-7} M epinephrine whereas phentolamine, a general alpha blocker, eliminated the effect of epinephrine. Thus, epinephrine in the fetal guinea pig lung appears to work through alpha-adrenergic receptors rather than beta-adrenoreceptors.

Experiments with specific alpha-adrenoreceptor antagonists have suggested that epinephrine stimulates two opposing mechanisms at high pharmacological concentrations while at physiological levels, epinephrine stimulates a single process. Studies with yohimbine (10^{-4} M), a specific α_2 -adrenoreceptor antagonist, and epinephrine at 10^{-5} M as well as 10^{-7} M suggest that α_1 -receptor stimulation causes a significant increase in lung fluid secretion. This is the first reported case where an increase in lung liquid production was obtained on a consistent basis. Tests with prazosin (10^{-5} M), a specific α_1 -receptor blocker, and epinephrine (10^{-5} M) appears to show that α_2 -receptor stimulation cause a significant reduction in lung liquid secretion. However, the lack of an effect of prazosin (10^{-5} M) at physiological levels of epinephrine (10^{-7} M) implies that α_1 -receptors function only at pharmacological levels. Therefore, at physiological levels of epinephrine, only α_2 -adrenoreceptors are activated to reabsorb lung

liquid and at pharmacological levels, two opposing mechanisms (α_1 and α_2 -adrenoreceptors) are stimulated to eliminate the effects of epinephrine.

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INTRODUCTION

During the 1700's, it was found that the fetal lung was filled with fluid (Winslow, 1787 quoted by Preyer, 1885). The fluid was thought to arise from the amnion and to be brought in by fetal respiratory movements (Preyer, 1885). However, Addison and How (1913) observed that the breathing movements could not be strong enough to draw the fluid into the lung. The possible mechanism of respiratory movements bringing fluid into the lungs was disproven by Windle et al. (1939). They concluded that the fetus did not exhibit breathing action, except under abnormal situations. Potter and Bohlender (1941) showed that the fetal lung was filled with fluid even when the trachea was blocked to prevent amniotic fluid from entering. Therefore, they concluded the lung fluid was not derived from the amniotic space. Whitehead et al. (1942) hypothesized the lung fluid was directly produced by the lung and that the fluid from the lung moved to the amniotic space. Jost and Policard (1948) confirmed the hypothesis using fetal rabbits. Their procedure involved decapitation at 19 days gestation (term is 31 days), and occlusion of the trachea. This experiment, the first in which fetal survival was achieved after surgery, showed the fetal lungs to be abnormally distended with liquid after 9 days. Therefore, they concluded that the liquid must be formed *in situ*. Also, Adams et al. (1963) showed differences in the composition between fetal lung fluid and amniotic fluid when collected simultaneously.

They found that the lung fluid (pH of 6.43) was more acidic than the amniotic fluid (pH of 7.06), and had a lower carbon dioxide level (4.4 mEq/litre compared to 18.0 mEq/litre for amniotic fluid).

Permeabilities of lung epithelium

Boyd et al. (1969) and Normand et al. (1971) investigated the permeabilities of pulmonary capillary and alveolar walls in the fetal sheep to a series of inert, uncharged, hydrophilic molecules. These molecules were radiolabelled and injected either into plasma or into lung fluid. Then timed samples were taken from pulmonary lymph, lung fluid, and plasma. To obtain rate constants for transport across the capillary endothelium and alveolar epithelium, the resulting concentration profiles were exposed to compartmental analysis. The two barriers were found to have very different permeabilities. The conclusions drawn from these experiments are:

- 1) The pulmonary capillary bed is sufficiently permeable to allow passage of all plasma constituents, including proteins, to the interstitial space and abluminal surface of the epithelium.
- 2) The lung epithelium is impermeable to macromolecules but is permeable to water and small solutes as a function of molecular size. It is also permeable to lipid-soluble substances such as respiratory gases. The impermeability to

water soluble substances bigger than a diffusion radius of 1.3 nm means that hydrophilic substances of this size and larger (albumin and blue dextran) can be used as volume markers for the lung lumen, allowing measurement of the rate of pulmonary fluid secretion or absorption.

Secretion of lung fluid

Lung volume

At 74 days, the earliest time in gestation at which tracer method measurements can be made (Olver et al., 1986), the fetal sheep lungs have been found to have 1 ml of liquid of which 30% is in the trachea. Between 74 and 96 days the lung volume distal to the trachea gradually increases from 3 to 10 ml/kg. For the next 10 days, there is a sharper rise due to rapid development of peripheral air spaces such that the volume doubles to 25 ml/kg (Olver et al., 1986). From then on there is only a slight increase in volume relative to body weight.

These lung secretions can be compared with absorptions that occur at birth. Humphreys et al. (1967) have found that perinatal absorption in sheep during the first 6 hr causes an average drop in lung weight of 27g/kg body weight. The pulmonary fluid volume of 30-33 ml/kg in the sheep fetus agrees well with estimates for the functional residual capacity of a newborn infant (Geubelle et al., 1959; Klaus

et al.,1962). This also agrees reasonably well with adult values of 34 ml/kg (Nelson, 1966). Therefore, it may be concluded that the lung volume per kilogram in the late fetal period is approximately the same as it is throughout postnatal life.

Fate of lung fluid

Some of the fluid that passes from the trachea to the oropharynx of the fetus is swallowed (Harding et al.,1984) but the remainder must contribute directly to the formation of amniotic liquid. Therefore, it is no surprise to find pulmonary surfactant in samples of amniotic fluid (Gluck et al., 1971). Although fetal urination is the major source of amniotic liquid, it has been suggested that the lung fluid can contribute from 25 to 50% of the daily amniotic production (Brown et al., 1983).

Lung development

The production of lung fluid is important for several reasons. The fluid provides a substantial source of amniotic fluid and can attain a secretion rate as high as 200 to 400 ml/day (fetal sheep) which is similar to fetal urine production (Barnes, 1976). The lung liquid has also been suggested to play an important role in salt and water regulation in the fetus (Cassin and Perks, 1982). Finally, the lung fluid production is essential for proper lung

development and growth (Potter and Bohlender, 1941; Alcorn et al., 1977).

The high resistance to liquid in the upper airway and the continual secretion of lung fluid exerts a slight but positive pressure maintaining expansion of the fetal lungs. Vilos and Liggins (1982) have stated that the pressure difference of 2 mmHg exists between the pulmonary lumen and the amniotic cavity whereas the pressure in the pleural space is only 0.2 mmHg below amniotic pressure. Therefore, most of the force maintaining lung expansion in the fetal sheep comes from lung secretion rather than from elastic recoil of the chest wall.

Alcorn et al. (1977) showed that long-term drainage of liquid from the lungs of fetal sheep caused virtual arrest of pulmonary tissue growth, whereas excessive lung distension after tracheal ligation led to hyperplasia. Similarly, removal of liquid from the right lung of fetal sheep, while liquid was retained in the left lung by bronchial occlusion, led to poor growth and decreased DNA content of the drained lung compared with a normal lung. On the other hand, the overexpanded lung displayed hyperplasia and increased DNA content (Moessinger et al., 1990). Fewell et al. (1983) have shown that pulmonary hypoplasia results in fetal sheep when a long-term tracheostomy is performed. This diminishes the resistance in the upper airway tract and allows lung fluid to freely drain into the amniotic space.

It has been well established in human infants that reduced volume of amniotic fluid (oligohydramnios) is associated with pulmonary hypoplasia (Perlman and Levin, 1974; Wigglesworth, 1987). However, there is no definite answer as to how the reduction in amniotic fluid volume affects pulmonary development. Harding *et al.* (1990) have shown that the forced flexion of the fetal body due to loss of amniotic fluid causes abdominothoracic compression. The forced flexion of the fetal body squeezes liquid from the easily compressible fetal lungs and hence cause hypoplasia as a result of lung volume reduction.

Lung hypoplasia, in human infants, is also associated with skeletal malformations and neuromuscular conditions. In thoracic skeletal dysplasia (Page and Stocker, 1982) and diaphragmatic paralysis due to Werdnig-Hoffman disease (Cunningham and Stocks, 1978), reduced lung volume could be responsible for the retardation in pulmonary growth. In summary, it appears that maintaining lung fluid volumes is important to normal growth and development.

Lung fluid composition

The clear or faintly opalescent lung fluid yields a clear colorless liquid containing 990 g H₂O/kg after centrifugation at 3×10^4 g (Strang, 1991). Adams *et al.* (1963) showed that comparisons of the lung fluid with the amniotic fluid revealed significant differences in pH, CO₂

levels, and ion concentrations. There were higher sodium (Na) and chloride (Cl) concentrations and lower potassium (K), protein, sugar and urea in lung fluid compared to the amniotic fluid. They also found that NaCl was excreted by the fetal lung. These results suggest that the lung fluid could be an ultrafiltrate of blood, with selective secretion or reabsorption by the lung. The different ion concentrations across the blood/pulmonary epithelial suggests an involvement of an ion pump.

Mechanism of lung secretion

The production of lung fluid due to an active pump rather than by simple filtration is necessary because of the enormous differences in ionic concentrations between the lung liquid and those expected in a plasma filtrate. Circulatory overload, sufficient to double pulmonary lymph flow, has been shown to have no effect on lung fluid production (Olver and Strang, 1974; Carlton *et al.*, 1989).

Olver and Strang (1974) investigated ion transport mechanism of lung fluid secretion by injecting labelled isotopes of several cations including Na^+ , K^+ , Ca^{2+} and halides such as Cl^- , Br^- , I^- into either the lung fluid or plasma of exteriorized fetal lambs. The one way fluxes and permeability constants were determined by following the changes in ion concentration in the fluids. The halides with K^+ and Rb^+ were found to be actively transported from

the plasma to the lumen of the lung. The chloride, however, was quantitatively the most important. The sodium and calcium ions passively diffused through the epithelium. A model was proposed using the active transport of chloride, from the plasma to the lumen, coupled to bicarbonate moving in the opposite direction causing an electrochemical gradient to allow passive movement of Na^+ and Ca^{2+} into the lung lumen. Finally, water enters in response to the osmotic gradient caused by the cations. Olver et al. (1975) found that the luminal side of the tracheal epithelium was electrically negative compared to the submucosal side due to the influx of Cl^- into the lumen. Olver et al. (1977) found similar results in the alveolar epithelium and in 1981 they showed that active chloride transport occurred in the fetal sheep lung by 84-87 days gestation (term = 147 days). Chloride appears to be a very important and integral part of the secretory mechanism.

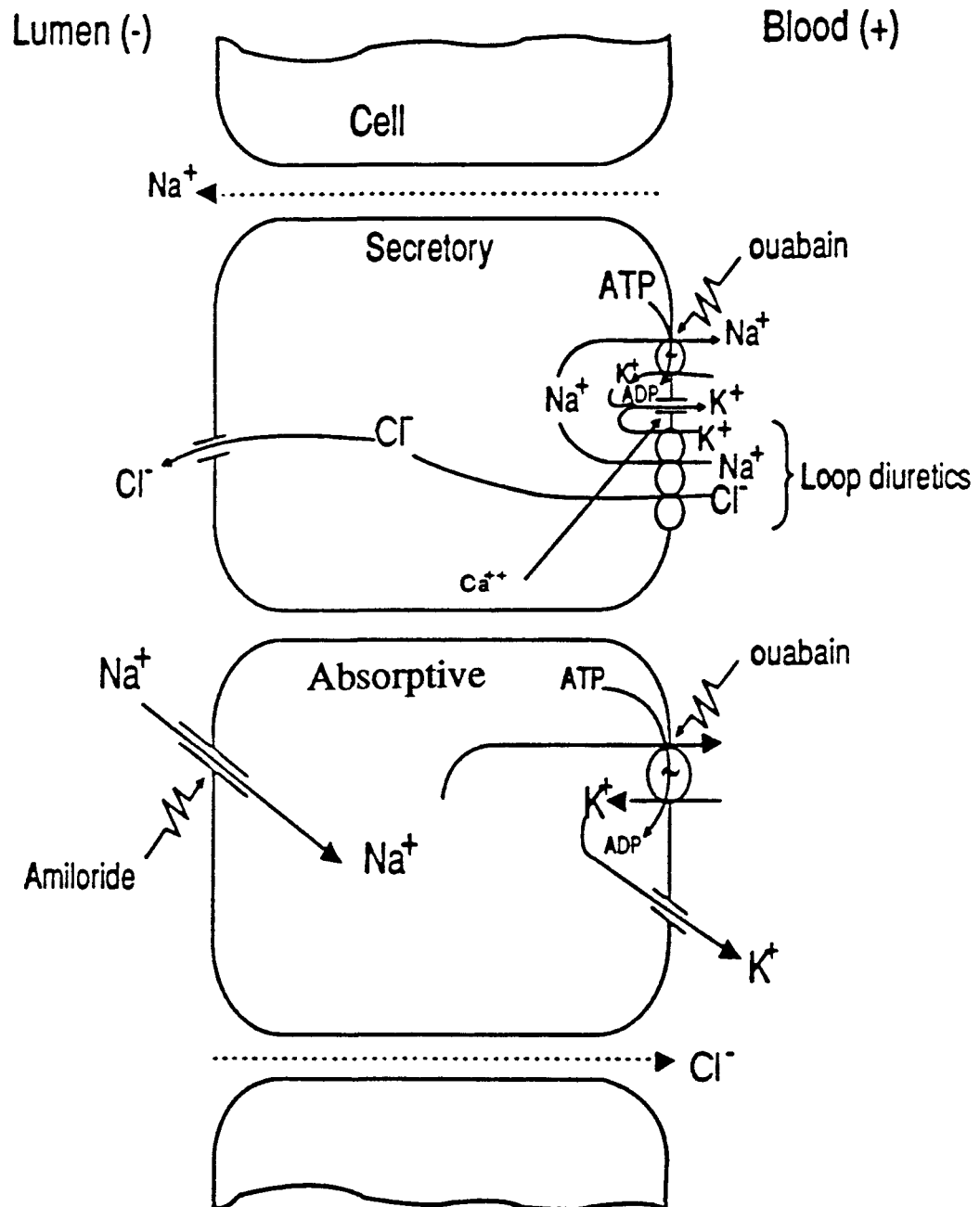
Further study on the Cl^- transport has been carried out using the shark rectal gland as a model (Gatzky, 1983). The chloride transport in the shark rectal gland is the same as in the adult canine trachea (Silva et al., 1977) and probably similar to the fetal alveolar cells (Widdicombe et al., 1979). Gatzky's model (Figure 1) involves active transport of sodium out of the epithelium along the basolateral membrane by a Na^+/K^+ ATP-dependent pump causing the interior of the cell to be electronegative relative to the blood or lumen. Sodium diffuses back through the

basolateral membrane because of a concentration gradient. Chloride enters against a gradient because of coupling to the sodium and the accumulation of Cl^- inside the cell forces the chloride to diffuse down the concentration gradient into the lung lumen. Sodium is removed from the cell and exchanged for potassium via Na^+/K^+ pump. Then potassium leaves passively through a channel on the basolateral membrane. The sodium can enter the cell from the lumen through a channel in the apical membrane to drive fluid reabsorption but the membrane is impermeable to sodium in the cell to lumen direction. The accuracy of the model is uncertain, however, there is strong evidence that Cl^- is a very important component of secretion.

Cassin et al. (1986) studied fetal lung secretion using loop diuretics and found that bumetanide and furosemide caused drastic reduction in secretion or stimulated fluid reabsorption in experiments using chronically catheterized near-term fetal lambs. Bumetanide and furosemide specifically inhibit chloride secretion on the apical side of the loop of Henle in the kidney (Imai, 1977). Similar results in guinea pigs were obtained by Thom and Perks (1990). These results show the importance of chloride in the mechanism of secretion.

Although cells responsible for secretion have not been identified, the cells must have Cl^- transport. Olver (1983) suspected chloride pumps on alveolar type II cells which

Figure 1. Gatzky's model of secretion and reabsorption in the lung epithelium.



line parts of the alveolar wall and are capable of considerable metabolic activity (Mason et al., 1982). Cultured alveolar type II cells showed dome formations, characteristic of transporting epithelium *in vitro* (Mason et al., 1982). Also, Alcorn et al. (1977) found constant removal of fetal lung fluid from the lamb increased the number of type II cells.

Reabsorption of lung fluid

The perinatal resorption of fluid which prepares the lung for air breathing has been shown to depend largely on the action of plasma epinephrine concentration on the ion transport. Epinephrine levels rise dramatically near term and have been shown to cause reabsorption of lung fluid in the fetus (Walters and Olver, 1978, Brown et al., 1980; Perks and Cassin, 1989). There is also evidence that vasopressin can suppress lung fluid secretion and that it could play a role in determining the changes at birth (Perks and Cassin 1985, 1989; Perks et al., 1993). Also, another mechanism has been observed, consisting of a change in epithelial solute permeability at the start of breathing, which is thought to facilitate passive reabsorption down the transpulmonary pressure gradient associated with ventilation and breath holding. There is speculation that the active reabsorption process initiated by epinephrine and the change in paracellular permeability are interrelated. Finally,

Na⁺-glucose cotransport has also been identified, but appears to be a quantitatively less important absorptive mechanism.

Effect of β -adrenergic receptor agonists and antagonists

Walters and Olver (1978) observed that intravenous infusion of isoproterenol (0.15-1.0 μ g/min) or epinephrine (0.2-3.0 μ g/min) caused reabsorption of lung fluid in the sheep fetus at gestations >129 days. This effect, which was immediately reversible on ceasing the infusion, could be blocked by a large dose of propranolol (0.2-3.0 mg/fetus), and since norepinephrine infused at a similar rate to epinephrine had no effect, it could be inferred that the reabsorptive response was mediated by β -adrenergic receptors. Using a preparation that allowed the measurement of lung fluid production by collection in a bag outside the uterus, Lawson et al., (1978) also showed that infusion of epinephrine at 3.3 μ g/min reduced the outflow of lung fluid in the sheep fetus. Reabsorption, however, could not be detected by this method. Rabbit fetuses which were injected with β -adrenergic agonists, showed less liquid content in lungs on delivery after a 3 hr interval than saline injected controls (Bergman et al., 1980; Enhorning et al., 1977; Lundell et al., 1988). However, Higuchi et al. (1987), McDonald et al. (1986) and Chapman et al. (1990) found that beta-adrenoreceptor antagonists did not affect liquid

secretion or reabsorption in the fetal lamb and fetal rabbit lung. Woods (1990) has found similar results in the fetal guinea pig lung.

The effects of epinephrine on lung fluid secretion and absorption appear to be dependent on gestational age of the fetus. In the chronically instrumented sheep fetus of < 130 days, intravenous infusion of epinephrine at 0.5 µg/min causes a decrease in secretion rate, whereas after that age it characteristically induces reabsorption, and this increases with advancing gestation (Brown et al., 1983). The epinephrine infusion rate of 0.5 µg/min appears to be definitely within physiological limits. It can be compared with experimental measurements by Comline and Silver (1961), who showed that during an episode of asphyxia or splanchnic nerve stimulation, the production of epinephrine from one adrenal gland was 0.3 µg/min at 130 days, increasing to 0.7 µg/min at 140 days. Similarly, when the epinephrine infusion rate of 0.5 µg/min was assessed in terms of plasma concentration rather than epinephrine output, it gave plasma levels within the physiological range. In the experiments of Brown et al. (1983), this rate of infusion increased the plasma concentration of epinephrine on average from 0.1 to 1.0 ng/ml (0.55-5.5 nM), which can be compared with values of 0.3-2.8 ng/ml (1.7-15 nM) observed during experimental asphyxia in the sheep fetus (Jones and Robinson, 1975) or with concentrations of 1.8-6.7 ng/ml (9.9-36 nM) reported in

umbilical cord plasma from the newly delivered human infant (Lagercrantz and Bistoletti, 1973).

Effects of labour

Brown et al. (1983) made measurements of lung fluid secretion and absorption rates during natural labour in the sheep and showed that reabsorption of pulmonary liquid took place in the latter part of labour. Reabsorption commenced during 150 to 50 min before delivery when the fetal plasma epinephrine concentration increased on average from the prelabour level of 0.09 ng/ml (0.49 nM) to 0.52 ng/ml (2.0 nM). The reabsorptive process became quite rapid in the 50 min before delivery, when the average epinephrine concentration was extremely high (6.9 ng/ml). The highest rates of about 29 ml/hr were observed during an artificially prolonged postnatal period before the onset of breathing. Therefore, the reabsorptive response during labour appeared to be explicable in terms of the adrenergic stimulus when the sheep fetus was close to term. However, such factors as vasopressin, a change in epithelial permeability, temperature and lung expansion after birth which might have a role in the reabsorption process, can not be ruled out.

Adenosine 3',5'-cyclic monophosphate effects

Olver et al. (1987) and Walter et al. (1990) have studied intracellular mediation of the β -adrenergic effect and its late gestational increase by adding 10^{-4} M dibutyryl-cAMP to fetal lung fluid. As with the β -adrenergic stimulus, cAMP produced a reabsorptive response in fetal sheep of > 130 days but only a decrease in secretion rate for younger fetuses. The response was delayed compared with that of epinephrine, being maximal 2 hr after addition to lung fluid, possibly due to the time required for diffusion from lung fluid to its effective site in the epithelial cell. The gestational pattern of increasing response toward term was closely similar to that seen with epinephrine infusion. This suggested that the maturation of the latter response depended not on the development of β -receptors but rather on gestational maturation of a component beyond cAMP in the intracellular signaling system responsible for transduction of the β -adrenergic stimulus to a reabsorptive response (Strang, 1991). Kindler et al. (1992) has also found that cAMP induces reabsorption of lung liquid in fetal guinea pigs.

Thyroid hormones and cortisol effects

It has been found that the maturation of the reabsorptive mechanism to epinephrine close to term is

determined by the synergistic action of triiodo-thyronine (T_3) and cortisol because these hormones exist in low plasma levels during early fetal life before showing a rise in late gestation similar to epinephrine.

Barker et al. (1990a) observed that a reabsorptive process could be induced when T_3 and cortisol were injected followed by epinephrine into the thyroidectomized fetal sheep at 116-120 days. Under normal conditions, epinephrine has no effect at this early gestational age. This injection provided plasma levels similar to end of term levels for these hormones (mean T_3 , 0.96 ng/ml, mean cortisol, 14 μ g/dl; epi, 0.5 μ g/min). Within 2 hours of injection a reabsorptive response was detectable. After 3 days of T_3 and cortisol the degree of absorption observed was similar to that of end of term. Barker et al., (1990b) also showed that T_3 alone did not have any significant results and therefore, suggested the synergistic relationship of T_3 and cortisol. The maturational effect was completely reversible, as within 24 hr of stopping the hormones the reabsorptive response to epinephrine had ceased (Barker et al., 1991). Furthermore, the addition of 10^{-4} M cyclohexamide, an inhibitor of protein synthesis at the ribosomal level, blocked the actions of T_3 and cortisol. Hence, it is likely that the synergistic actions depended on synthesis of a protein component of the signaling system in lung epithelium at some point between cAMP and the effector of the reabsorptive response (Barker et al., 1991).

Epinephrine induced absorption

The mechanism of epinephrine induced reabsorption in fetal sheep was studied using measurements of ion fluxes, electrochemical potentials, and responses to amiloride by Olver et al. (1986). The measurements of electrical potential differences (PD) between lung fluid and plasma showed an immediate, reversible increase in luminal electronegativity in response to intravenous epinephrine. In studies that exhibited reabsorption, the increase in PD averaged 1.8 mV (0.3SE, n=21). If the reabsorption had been consequent only on suppression of the Cl^- pump, the PD should have decreased to zero, and if the direction of Cl^- transport had reversed, the potential should have become positive on the luminal side. The increase in electronegativity, which actually took place in response to epinephrine, was the result expected if the β -adrenergic agonist induced active Na^+ transport from the luminal side of the epithelium toward the interstitium. Any contribution of a decrease in capillary hydrostatic pressure as a factor favouring reabsorption could be discounted because infusion of epinephrine at 0.5 $\mu\text{g}/\text{min}$ into a peripheral vein was found to have no detectable effect on the pulmonary circulation (Olver et al., 1986).

One-way Na^+ and Cl^- fluxes were measured by Olver et al. (1986). One-way fluxes were determined by compartmental analysis of tracer concentration curves after introduction

of ^{24}Na and ^{36}Cl into lung fluid. The flux ratios were then compared with the expected fluxes for passive transfer predicted by Ussing's equation. Data from Olver et al. (1986) shows that epinephrine-induced reabsorption was associated with an increase in the one-way flux of Na^+ from lumen to interstitium but with no change in the opposite Na^+ flux. There was also an increase in Cl^- flux from lumen to interstitium with no change in one-way Cl^- flux toward the lumen. The calculated transport potential for Na^+ changed significantly during epinephrine infusion from a negligible value of 0.6 to -8.8mV (the negative sign indicating active transport of Na^+ from the luminal side). At the same time, the transport potential for Cl^- decreased in response to epinephrine from 21 to 2.2 mV, which indicated there was virtually no active Cl^- transport to the lumen. However, these effects of epinephrine were blocked by the addition of amiloride on the luminal side of the epithelium. Its addition to lung fluid before starting the epinephrine infusion completely inhibited the reabsorptive response and abolished the accompanying changes in ion flux and transport potential. The blocking action was also demonstrated by adding this substance to pulmonary fluid after starting the epinephrine infusion, when it had the effect of stopping reabsorption and initiating secretion at the pre-epinephrine rate despite continuation of the epinephrine infusion. Fifty percent inhibition of the reabsorptive response to epinephrine(K_1) was obtained at an amiloride concentration

of 4.3×10^{-4} M, which is within the range characteristic for its specific Na^+ channel blocking effect. In isolated epithelia exposed to very low $[\text{Na}^+]$, the K_1 for its selective action on Na^+ channels is lower, 10^{-7} to 10^{-8} M, but in the presence of high $[\text{Na}^+]$, its K_1 is a little higher, for example, 10^{-6} M in rat colon (Edmonds, 1981). Because epinephrine induced reabsorption was inhibited by amiloride in the micromolar range, its effect could be attributed to its Na^+ channel blocking action. The inhibitory effect of amiloride in the epinephrine-stimulated lung showed that absorption depended on the presence of open Na^+ channels. In the absence of adrenergic stimulation, however, amiloride had no effect or increased secretion minimally, which indicated that the channels are normally closed in the secretory state. Indeed, it can be argued that all the actions of epinephrine on ion transport in this epithelium are explicable by its action in opening apical membrane Na^+ channels and that the open or closed state of these channels determines whether the fetal lung, at any particular time, is secretory or reabsorptive (Strang, 1991).

Electrolyte transport changes during the perinatal stage have also been studied in isolated type II alveolar cells from fetal, newborn, and adult rabbits (Bland and Boyd, 1986; Chapman et al., 1990). The sodium pump activity, measured as the ouabain-inhibitable component of ^{86}Rb uptake, was shown to be 3 to 4 times greater in cells

obtained from newborn rabbits delivered after a period in labour than from rabbits delivered by hysterotomy before labour (Bland and Boyd, 1986). After the neonatal period there was a further increase in pump activity to an adult level, 2-3 times greater than the neonatal value and 10 times greater than the fetal value. By measurement of [^3H]ouabain binding, it was shown that the late postneonatal increase in Na^+ transport was associated with a rise in the number of pump sites on type II cells, whereas no difference was found between the numbers of pump sites on fetal and early postnatal cells (Chapman *et al.*, 1990). Therefore, the immediate perinatal increase in Na^+ pump activity period was attributed to increased turnover of ATPase rather than to an increased amount of the enzyme. Because the perinatal change in electrolyte transport appears to be associated with the opening of apical membrane Na^+ channels, it follows that the early perinatal changes detected in these experiments were probably secondary to increased access of Na^+ to basolateral membrane pump sites. According to this interpretation, it should be the opening of apical membrane Na^+ channels that initiates and determines the perinatal changes in electrolyte transport (Chapman *et al.*, 1990).

β -adrenergic agonists stimulate Cl^- secretion in most Cl^- secreting epithelia, including that covering the conducting airways of many fetal and adult mammals. Therefore, the effects of epinephrine in suppressing Cl^- transport is a very unusual characteristic of the fetal

pulmonary epithelium. However, the effects of epinephrine on both Na^+ and Cl^- transport and the blocking action of amiloride provide a model for secondary Na^+ -dependent Cl^- transport (Frizzell et al. 1979). To account for the amiloride-blockable response to epinephrine, it is suggested that the alveolar epithelial cells responsible for Cl^- transport in the mature fetal lung are equipped with reversibly opening Na^+ channels in their apical membranes. When these channels are closed in the resting state, the basolateral Na^+ pump drives Cl^- to the lumen. However, when the channels open in response to a rise in cAMP, Na^+ enters the cell and gains access to the basolateral Na^+ pump. The entry of positive charges into the epithelial cell would be expected to depolarize its apical membrane and hence decrease the potential for Cl^- extrusion to the lumen. This could explain both the initiation of active Na^+ transport and inhibition of Cl^- transport.

Role of vasopressin

Experimental studies suggest that arginine vasopressin (AVP) can influence lung secretion in the fetal sheep or goat.

AVP is known to increase water and solute permeability as well as Na^+ transport in several epithelial barriers (Sharp, 1972). Also, AVP levels increase dramatically in its plasma concentration during parturition (Stark et al.,

1979). Therefore, this hormone might have a very significant role in the perinatal changes in lung fluid dynamics. Perks and Cassin (1982) reported that high doses of AVP in a goat infused into the lung fluid at 64 mU/ml produced a short-lived reabsorption.

Wallace et al., (1990) measured the effects of intravenous infusion of AVP on lung fluid secretion at different gestational ages in the fetal sheep. The infusion increased the plasma AVP to a level of 849 pg/ml which is the normal range during labour (Stark et al., 1979). The effect of a 2 hr infusion of AVP depended on gestational age. Fetuses up to 135 days had no effect, whereas between 135 and 140 days, they produced a 41% reduction, and after 140 days, they produced a 78% reduction in secretion rate. In fetuses not undergoing labour, AVP showed no reduction in secretion.

AVP has also been found to reduce lung fluid secretion rates in the guinea pig (Perks et al., 1993), sheep (Perks and Cassin, 1985) and goats (Perks and Cassin, 1989).

These studies did not show that AVP causes reabsorption but it reduces the secretion rate with increasing age and its concentration in the plasma rises during labour. Therefore, AVP could play a role in the secretory and reabsorptive processes.

STATEMENT OF THE PROBLEM

Epinephrine levels in the fetus of many animals as well as humans have been found to increase considerably near term and epinephrine has been shown to work through beta-adrenoreceptors in the fetal sheep to induce reabsorption of fetal lung fluid. However, recent work by Woods (MSc thesis, 1990) appears to show that epinephrine works through alpha-adrenoreceptors instead of beta-adrenoreceptors in the fetal guinea pig lung. Woods (1990) found that the lung had a dose dependent response to epinephrine but at high pharmacological levels the lung did not appear to respond. This observation was quite unexpected and the present study tried to further investigate this anomaly. We postulated that at very high concentrations of epinephrine, two opposing mechanisms became stimulated to eliminate any effects on lung liquid secretion. Woods (1990) has shown that alpha-adrenoreceptors are most likely responsible for the opposing mechanisms. Alpha-adrenoreceptors are subclassed into α_1 and α_2 adrenoreceptors with α_1 receptors opposing the actions of α_2 receptors. This study tested the possibility of epinephrine stimulating two opposing alpha-adrenoreceptors at pharmacological concentrations.

MATERIALS AND METHODS

Animals

Pregnant albino guinea pigs (*Cavia porcellus*) of an inbred departmental stock were given food and water *ad libitum* (guinea pig chow, Ralston-Purina; supplemented by fresh vegetables and vitamin C). Fetuses used in the experiments were between the ages of 58-65 days with term being at about 67 days gestation. The majority of gestational ages were calculated from previous delivery dates, as guinea pigs enter estrus immediately following delivery. However, if this information was not known, gestational ages were estimated from the average fetal weight and the size of the litter, according to the methods of Ibsen (1928) but using data based on 400 fetuses of our own stock.

Basis of the Method

Measurements of lung fluid production were based on an impermeant tracer technique using Blue Dextran 2000 (Pharmacia, Dorval, Que.: Stokes's radius, 270 angstroms (1 angstrom=0.1nm); radius of gyration, 380 angstroms; molecular mass, 2 000 000 Daltons). This technique has been used by Normand and co-workers (1971), Martins and co-workers (1975) and Liu and Chiou (1981). This method has also been used in previous studies of lung fluid secretion in fetal sheep and goats *in vivo*, and was checked with the

simultaneous use of ^{125}I -labelled albumin (Cassin and Perks 1982; Perks and Cassin 1985a, 1985b, 1989).

Surgical Procedures

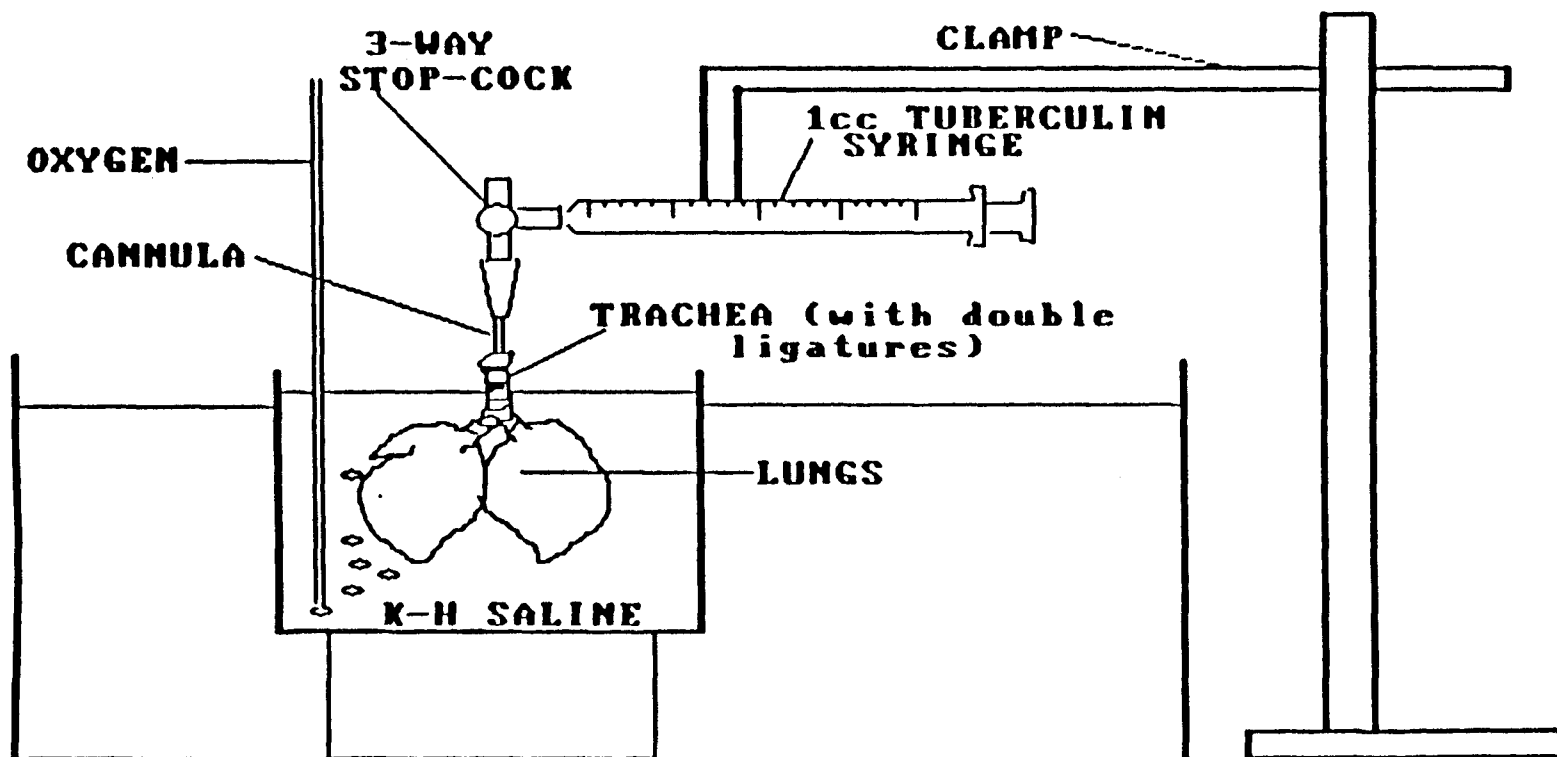
Using halothane, pregnant guinea pigs of late gestation were anesthetized until the corneal reflex was extinguished. The fetuses, with the amnion intact, were extracted by Caesarean section. The fetal lungs and trachea were exposed using a mid-line incision along the thorax and the trachea was ligated rostrally. Just below the ligature a small incision was made on the ventral surface of the trachea, through which the trachea was cannulated with 1.5-2.0 cm polyethylene tubing (PE 50; Intramedic, Clay Adams, Parsippany, N.J.). The cannula was attached to a 1.0 ml tuberculin syringe via an 18G hypodermic needle and a 3-way stop-cock (K75, Pharmaseal, Puerto Rico). The cannula was placed just above the bifurcation of the bronchi and secured with two ligatures. The heart and cannulated lungs were then carefully excised, and following the separation of the heart, the isolated lungs were suspended in a 100 ml bath containing Krebs-Henseleit saline (pH 7.4). The saline was well oxygenated with 95% O_2 /5% CO_2 and maintained at a constant temperature of 37°C to further mimic optimum *in vivo* conditions. These surgical procedures took 3 to 4 minutes during which the lungs were kept moist and warm by frequent washes with saline at 37°C .

Experimental Procedures

Lung fluid (0.1-0.8 ml) was drawn into the syringe of the cannula assembly (Figure 2) and redistributed to the upper cup of the 3-way stop-cock. Blue Dextran 2000 (0.1 ml; prepared as 50mg/ml in 0.9% NaCl) was then added to the cannula assembly via the upper cup and thoroughly mixed with the lung fluid in the syringe (1701 NCH gas-tight fixed volume syringe; Hamilton Co., Reno, Nevada) before being passed into the lungs. A 30 minute interval was allowed for equilibration, during which time the lung fluid was "mixed" (by withdrawing lung fluid into the syringe and then returning it to the lungs) at 5 minute intervals to ensure proper distribution of Blue Dextran throughout the lungs. In addition, the saline bath was changed after 15 minutes and again after 30 minutes. After dye equilibration, the lungs were maintained for 3 hours with the supporting saline being renewed at the start of each hour. Samples of lung fluid (10 μ l) were taken thereafter at 10 minute intervals, and lung fluid was "mixed" at 5 minute intervals to ensure even distribution of dye. The samples were placed in polyethylene micro test tubes (250 μ l Eppendorf C3515-7, Brinkman Instruments Ltd., Rexdale, Ont.), and diluted by a factor of 20 with 190 μ l distilled water. The samples were sealed, well mixed with a vortex (Vortex-Genie, Fisher Scientific, N.Y.) and centrifuged at 250 G for 10 minutes (clinical centrifuge,

Figure 2. *In vitro* preparation used for the maintenance of the guinea pig lung.

The bath contains Krebs-Henseleit saline maintained at 37°C and bubbled with 95% O₂/ 5% CO₂.



Model CL, International Equipment Co., Needham Heights, Mass.). The supernatant was removed by syringe, and the concentration of Blue Dextran in each sample was estimated using spectrophotometry (Guilford 250, Oberlin, Ohio and Beckman DU-8 UV-Visible Computing Spectrophotometer; 250 μ l quartz microcells, Type 10972, NSG Precision Cells Inc., Farmington, N.Y.; wavelength=620 nm). The 3 hour experiment followed an A/B/A design: (1) saline alone, (2) treatment in saline and (3) saline alone. Therefore, the samples have a resting rate during the first hour, a treatment rate during the second hour and a recovery rate in the third hour.

Epinephrine hydrochloride (Adrenalin, Parke-Davis Inc.) was tested at concentrations of 10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} M during the second hour of the experiment. The fetal lungs were also exposed to epinephrine at 10^{-7} M with 10^{-6} M propranolol (Sigma Chemical Co.) to test effects of beta-adrenoreceptors. Phentolamine (Sigma Chemical Co.) at 10^{-6} M was then tested with 10^{-7} M epinephrine to test the role of alpha-adrenoreceptors. Yohimbine (Sigma Chemical Co.) at 10^{-4} M was tested with 10^{-7} M and 10^{-5} M epinephrine while prazosin (Sigma Chemical Co.) at 10^{-5} M was also tested with both concentrations of epinephrine to examine the role of specific subtypes of alpha-adrenoreceptors. Controls for the blockers were also done.

Quantification of Results and Statistical Methods

Fluid production rates were determined from the change in concentrations of Blue Dextran (Cassin and Perks, 1982; Perks and Cassin, 1989). Total volumes (in ml) of fluid present in the lungs were calculated, taking into account the addition of Blue Dextran at the onset of experimentation and making appropriate sequential adjustments every 10 minutes for the removal of fluid and Dextran during sampling. Total volumes (expressed as a percentage relative to sample #6 which is set at 100%) were plotted against time, and slopes over 1 hour intervals were calculated by linear regression, fitted by the method of least squares (Steel and Torrie(1970); Apple II computer). For each hour, the fluid production rates in ml/kg·h were determined as the change in total volume (in ml) over time relative to the weight of the fetus. The significance of changes in rate were estimated from changes in slope, analyzed by two-way analysis of variance (ANOVA) and followed by a contrast test (Zar 1984). Statistical significance was accepted at $P < 0.05$. Combined data were obtained by averaging the total volumes (in %) at each 10 minute interval for a given set of experiments, as well as averaging the rates for each hour. Averages are reported with standard errors for all combined results.

RESULTS

A. Studies of the effects of epinephrine on lung fluid secretion

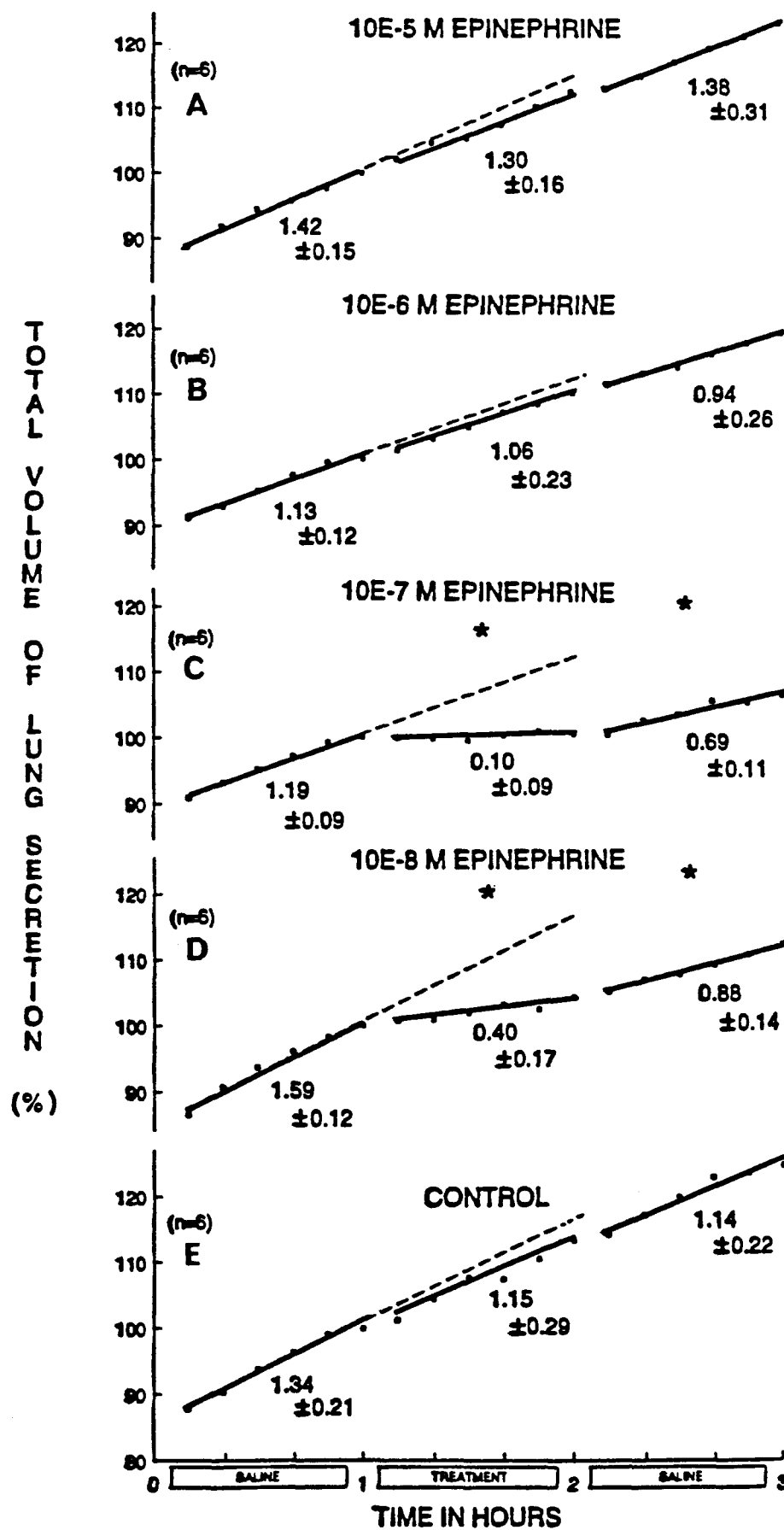
To test the effects of epinephrine, various concentrations were used: 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M (Figure 3). These concentrations covered the top of the dose/response curve for epinephrine, where reabsorptive effects were expected to be maximal, however, at pharmacological levels the response to epinephrine disappeared. The objective was to confirm independently the unexpected observations of Woods (1990) and this was accomplished as follows:

Epinephrine at 10^{-5} M (Figure 3A)

Six fetal lungs (average fetal body weight= 76.36 ± 4.80 grams and average gestational age of 61 ± 1 days) were subjected to 10^{-5} M epinephrine during the second hour of the experiment. Before the start of the second hour the bath was changed for fresh Krebs' saline and epinephrine. The rates obtained were 1.42 ± 0.15 , 1.30 ± 0.16 , and 1.38 ± 0.31 ml/kg·hr for the first, second and third hours respectively. There were no significant differences between the rates after analysis using two way ANOVA (significance accepted at $p < 0.05$).

Figure 3 The effect of epinephrine (epi) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 30 fetuses, 59-65 days gestation, with an average body weight of 80.61 ± 2.76 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-5} M epi (n=6); (B) 10^{-6} M epi (n=6); (C) 10^{-7} M epi (n=6); (D) 10^{-8} M epi (n=6); (E) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate (significance accepted at $p < 0.05$).



Epinephrine at 10^{-6} M (Figure 3B)

Six fetal lungs (average body weight= 86.24 ± 3.23 grams and average gestational age of 61 ± 1 days) were exposed to 10^{-6} M epinephrine during the second hour. Rates of 1.13 ± 0.12 , 1.06 ± 0.23 , and 0.94 ± 0.26 ml/kg·hr were obtained and no significant differences among the rates were determined using two way ANOVA.

Epinephrine at 10^{-7} M (Figure 3C)

Fetal lungs (n=6, average weight= 88.23 ± 7.98 g, average age of 61 ± 2 days) were subjected to 10^{-7} M epinephrine during the second hour. Two way ANOVA revealed significant differences between the rates: 1.19 ± 0.09 , 0.10 ± 0.09 , and 0.69 ± 0.11 ml/kg·hr. By a test of contrast, the first hour was different from the second and third hour (significance accepted at $p < 0.05$) (marked by asterisks). There was a $91.60 \pm 9.74\%$ fall in secretion rate during the second hour. Figure 3C shows a dramatic effect of epinephrine during the second hour.

Epinephrine at 10^{-8} M (Figure 3D)

Epinephrine (10^{-8} M) was introduced to fetal lungs (n=6, average weight= 74.62 ± 4.31 g, average age of 60 ± 1 days)

during the second hour. The rates obtained were 1.59 ± 0.12 , 0.40 ± 0.17 , and 0.88 ± 0.14 ml/kg·hr. There was a $74.84 \pm 11.63\%$ fall in secretion in the second hour and two way ANOVA revealed significant differences between the rates. Scheffé's test revealed that the first and second hour rates differed significantly.

Controls (Figure 3E)

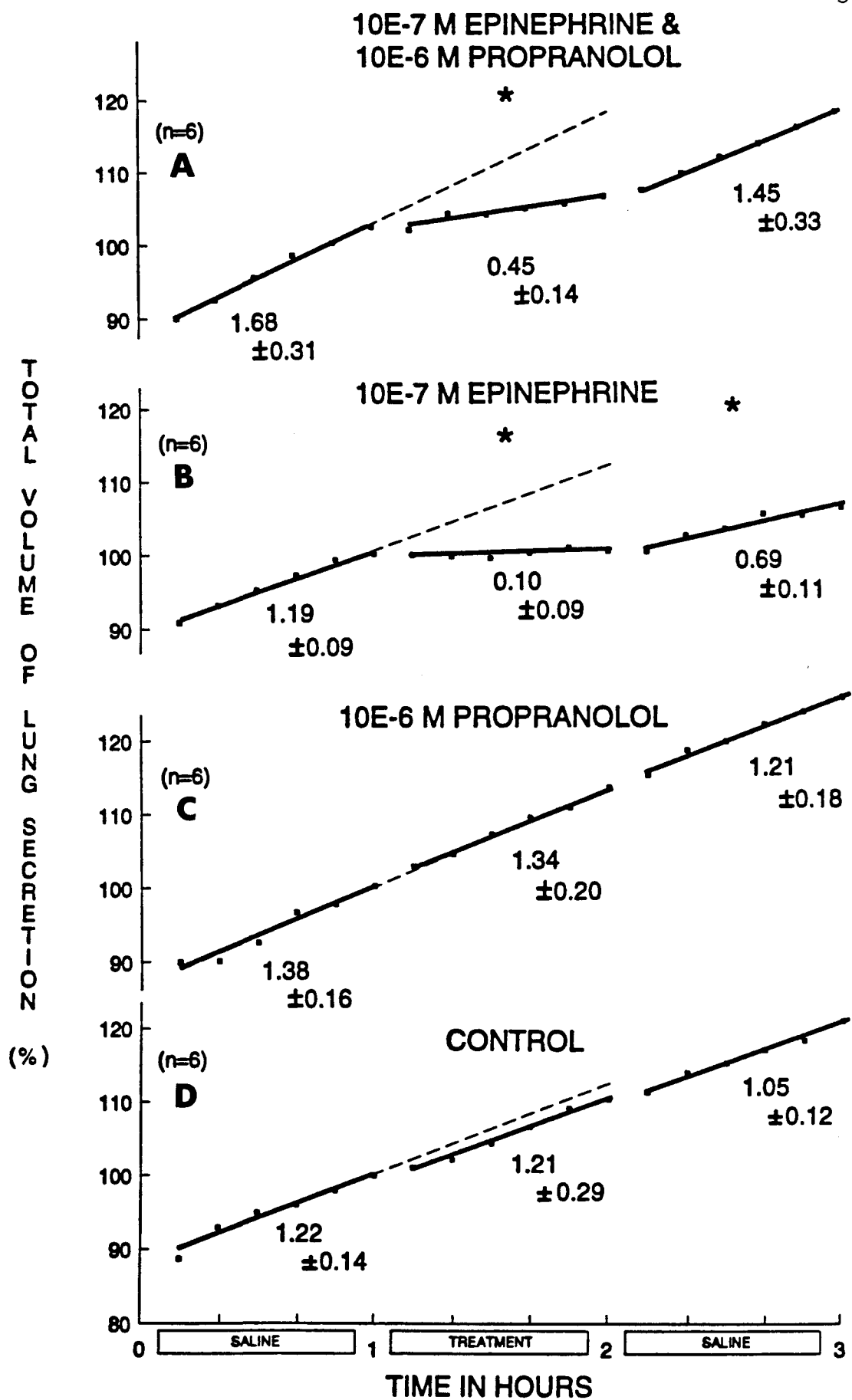
Control experiments were carried out on lungs of fetuses (n=6, average weight= 77.59 ± 3.60 g, average age of 63 ± 1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.34 ± 0.21 , 1.15 ± 0.29 , and 1.14 ± 0.22 ml/kg·hr.

B. Studies of the effects of α and β receptor antagonists on responses to epinephrine at physiological levels

Earlier studies by Woods (1990) suggested that alpha and not beta receptors were responsible for the effects of epinephrine on the fetal guinea pig lung. The following experiments involving general alpha and beta antagonists were carried out at 10^{-7} M epinephrine to confirm Woods' (1990) results because maximal effects were observed at physiological levels of epinephrine (Jelinek and Jensen, 1991).

Figure 4. The effect of epinephrine (epi) and propranolol (pro) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-65 days gestation, with an average body weight of 77.40 ± 5.20 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-7} M epi/ 10^{-6} M pro (n=6); (B) 10^{-7} M epi (n=6); (C) 10^{-6} M pro (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



Epinephrine (10^{-7} M) and Propranolol (10^{-6} M) (Fig. 4)

Although epinephrine activated fluid reabsorption in sheep via beta receptors, earlier work had suggested that these receptors do not operate in the guinea pig (Woods, 1990). Initial studies at physiological levels of epinephrine used the beta-antagonist propranolol to confirm these findings. When lungs from fetuses ($n=6$, average weight = 76.00 ± 4.88 g, average age of 60 ± 1 days) were exposed to epinephrine at 10^{-7} M with 10^{-6} M propranolol, the secretion rate fell significantly (two way ANOVA) during the second hour (Fig. 4A). The rates were 1.68 ± 0.31 , 0.45 ± 0.14 , and 1.45 ± 0.33 ml/kg·hr. Therefore, propranolol could not block the effects of 10^{-7} M epinephrine which caused a significant decrease in secretion rate (Figure 4B) when used alone.

Propranolol (10^{-6} M) (Fig. 4C)

The rates obtained with propranolol were 1.38 ± 0.16 , 1.34 ± 0.20 , and 1.21 ± 0.18 ml/kg·hr and two way ANOVA showed no significant differences among the rates. Propranolol had no apparent actions on the lungs ($n=6$, average weight = 76.01 ± 2.90 g, average age of 60 ± 1 days).

Controls (Figure 4D)

Control experiments were carried out on lungs of fetuses $n=6$, average weight= 70.35 ± 3.66 g, average age of 61 ± 1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.22 ± 0.14 , 1.21 ± 0.29 , and 1.05 ± 0.12 ml/kg·hr.

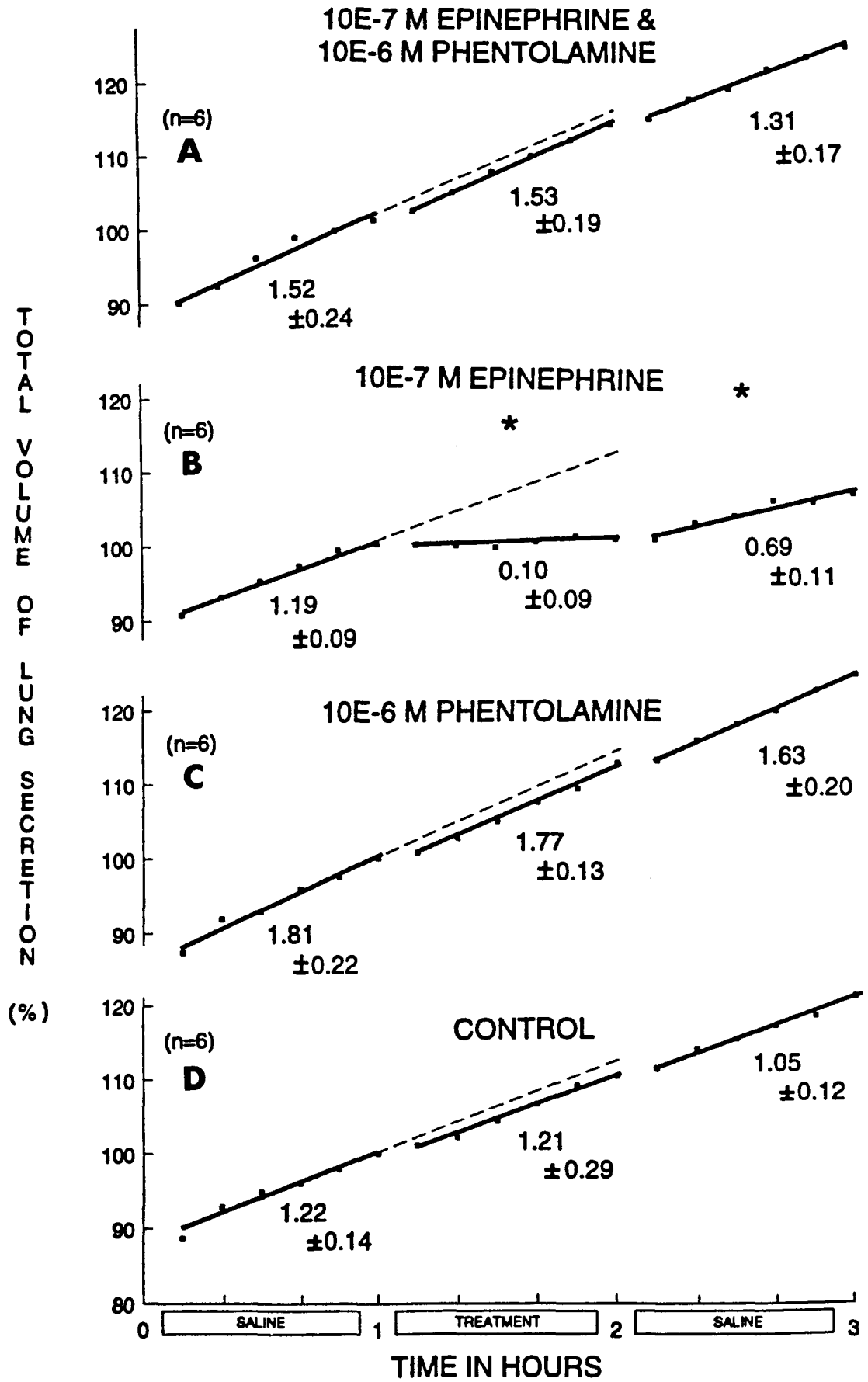
The above results suggest that epinephrine does not work through beta receptors to cause reductions in fluid production or liquid reabsorption.

Epinephrine (10^{-7} M) and Phentolamine (10^{-6} M) (Fig. 5)

Before testing the more specific α_1 and α_2 antagonists, the more general alpha-adrenergic receptor blocker, phentolamine was used. 10^{-7} M epinephrine and 10^{-6} M phentolamine produced rates of 1.52 ± 0.24 , 1.53 ± 0.19 , and 1.31 ± 0.17 ml/kg·hr. Two way ANOVA showed no significant differences in rates. Therefore, phentolamine appears to block the actions of epinephrine on the lungs of fetuses ($n=6$, average weight= 78.73 ± 2.97 g, average age of 60 ± 1 days). This suggests that epinephrine works through alpha-adrenergic receptors. In contrast, epinephrine alone at 10^{-7} M caused significant reductions in secretion rates (Figure 5B).

Figure 5. The effect of epinephrine (epi) and phentolamine (phe) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-65 days gestation, with an average body weight of 78.25 ± 3.75 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-7} M epi/ 10^{-6} M phe (n=6); (B) 10^{-7} M epi (n=6); (C) 10^{-6} M phe (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



Phentolamine (10^{-6} M) (Fig. 5C)

Phentolamine appears to have no significant effect on secretion rates (ANOVA). The rates were 1.81 ± 0.22 , 1.77 ± 0.13 , and 1.63 ± 0.20 ml/kg·hr for the lungs of fetuses (n=6, average weight= 75.68 ± 5.04 g, average age of 61 ± 1 days). The results suggest that phentolamine alone has no effect on lung fluid production.

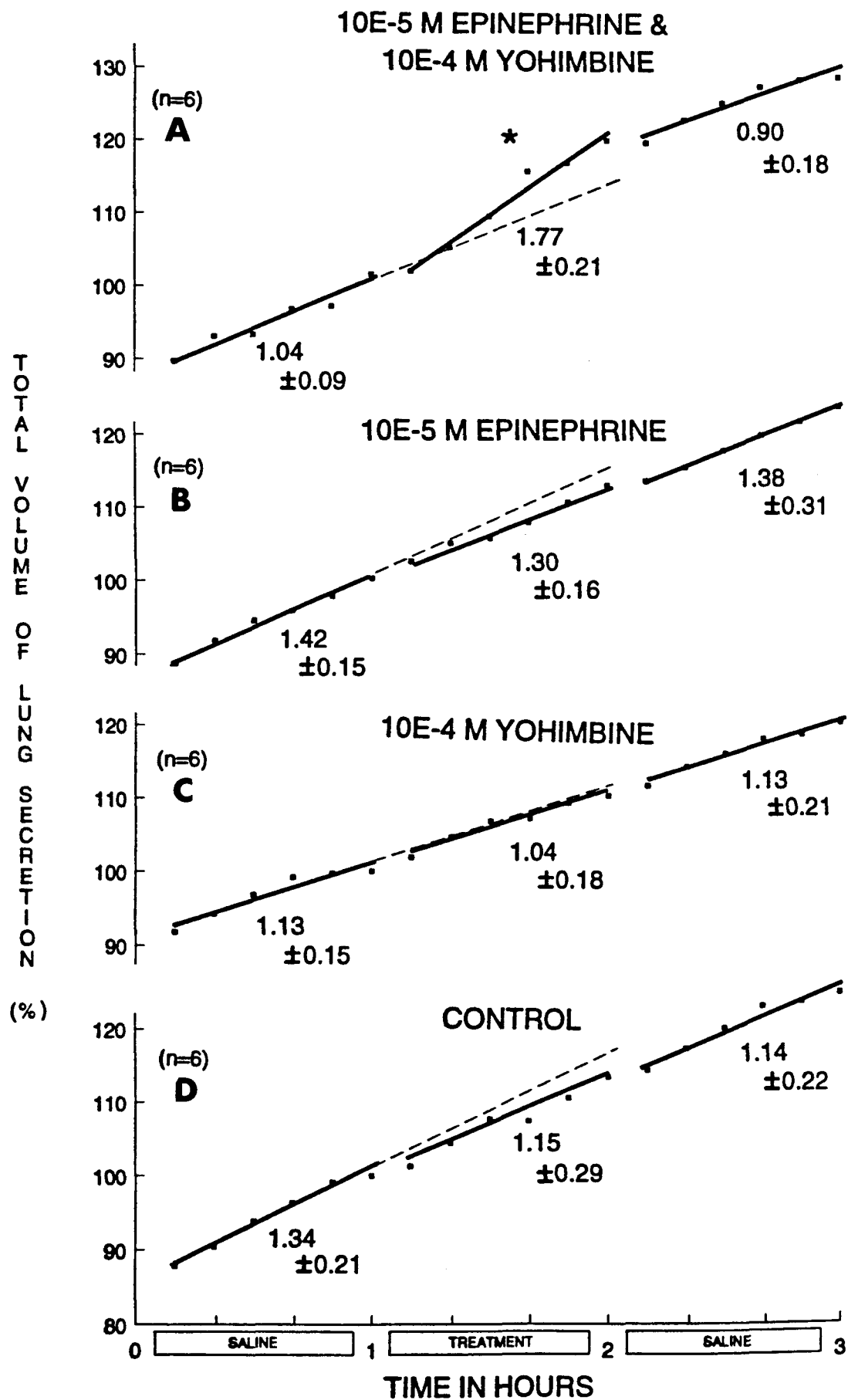
Controls (Figure 5D)

Control experiments were carried out on lungs of fetuses (n=6, average weight= 70.35 ± 3.66 g, average age of 61 ± 1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.22 ± 0.14 , 1.21 ± 0.29 , and 1.05 ± 0.12 ml/kg·hr.

The data appears to show that epinephrine is operating via alpha receptors and not beta receptors in the fetal guinea pig lung. This confirms data collected by Woods (1990).

Figure 6. The effect of epinephrine (epi) and yohimbine (yoh) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-64 days gestation, with an average body weight of 81.99 ± 4.50 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-5} M epi/ 10^{-4} M yoh (n=6); (B) 10^{-5} M epi (n=6); (C) 10^{-4} M yoh (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



C. Studies of the effects of alpha-receptor antagonists on responses to high levels of epinephrine (10^{-5} M).

These studies were designed to determine whether the failure of high concentrations of epinephrine to reduce fluid production was due to antagonism between α_1 and α_2 receptors.

Epinephrine (10^{-5} M) and yohimbine (10^{-4} M) (Fig. 6)

To test the effects of epinephrine on α_1 -adrenoreceptors yohimbine, a specific α_2 -adrenoreceptor antagonist, was used to eliminate possible opposing effects. Yohimbine at 10^{-4} M and 10^{-5} M epinephrine produced a significant increase (two way ANOVA) in secretion rate in the second hour compared to the first hour rate. The following rates were obtained 1.04 ± 0.09 , 1.77 ± 0.21 , and 0.90 ± 0.18 ml/kg·hr. The lungs displayed no significant response to 10^{-5} M epinephrine by itself (Figure 6B). The fetuses used had an average weight of 95.44 ± 2.81 grams, average age of 62 ± 1 days gestation, and an n=6.

Yohimbine (10^{-4} M) (Fig. 6C)

Lungs (n=6, average weight= 78.31 ± 4.64 g, average age of 61 ± 1 days) treated with yohimbine showed rates of 1.13 ± 0.15 ,

1.04±0.18, and 1.13±0.21 ml/kg·hr . No differences between the rates were detected by two way ANOVA.

Controls (Figure 6D)

Control experiments were carried out on lungs of fetuses (n=6, average weight=77.59±3.60 g, average age of 63±1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.34±0.21, 1.15±0.29, and 1.14±0.22 ml/kg·hr.

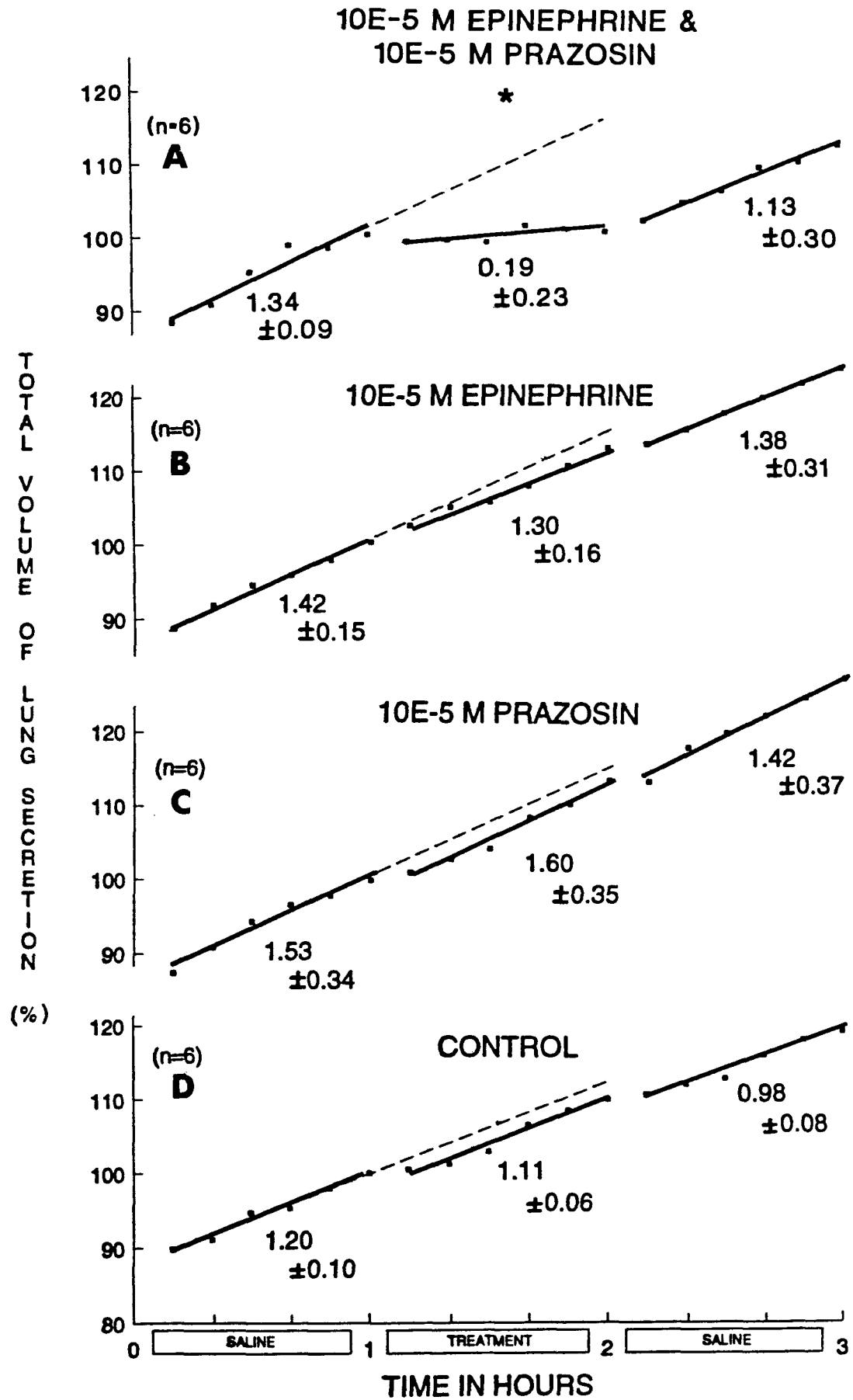
The results above suggest that high levels of epinephrine can stimulate secretion via α_1 receptors when α_2 receptors are blocked.

Epinephrine (10^{-5} M) and prazosin (10^{-5} M) (Fig. 7)

Prazosin, a specific α_1 -adrenoreceptor blocker, was used with epinephrine to test the effects of α_2 -adrenoreceptor stimulation alone. Prazosin at 10^{-5} M and 10^{-5} M epinephrine caused a dramatic decrease in secretion rate during the second hour (Figure 7A). The following rates were obtained 1.34±0.09, 0.19±0.23, and 1.13±0.30 ml/kg·hr. Two way ANOVA shows significant differences between the rates. Epinephrine alone at this concentration (10^{-5} M) appeared to have no effect on the lungs (Fig. 7B).

Figure 7. The effect of epinephrine (epi) and prazosin (pra) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-62 days gestation, with an average body weight of 75.43 ± 2.84 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-5} M epi/ 10^{-5} M pra (n=6); (B) 10^{-5} M epi (n=6); (C) 10^{-5} M phe (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



The fetuses used had an average weight of 80.11 ± 4.10 grams, average age of 61 ± 1 days gestation, and an $n=6$.

Prazosin (10^{-5} M) (Fig. 7C)

During studies of prazosin alone, rates in successive hours were 1.53 ± 0.34 , 1.60 ± 0.35 , and 1.42 ± 0.37 ml/kg·hr from the lungs of fetuses ($n=6$, average weight= 78.00 ± 4.01 g, average age of 60 ± 1 days). No differences between the rates were detected by two way ANOVA.

Controls (Figure 7D)

Control experiments were carried out on lungs of fetuses ($n=6$, average weight= 67.23 ± 4.74 g, average age of 60 ± 1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.20 ± 0.10 , 1.11 ± 0.06 , and 0.98 ± 0.08 ml/kg·hr.

The results suggest that epinephrine causes reabsorption or a strong decrease in secretion via α_2 receptors if α_1 receptors are blocked.

General conclusion to studies at high levels of epinephrine

The effects of α_1 and α_2 antagonists suggest that the failure of high levels of epinephrine to induce reduction in fluid production is due to joint stimulation of opposing stimulatory and inhibitory systems acting through α_1 and α_2 receptors respectively.

D. Studies of the effects of alpha-receptor antagonists on responses to physiological levels of epinephrine (10^{-7} M).

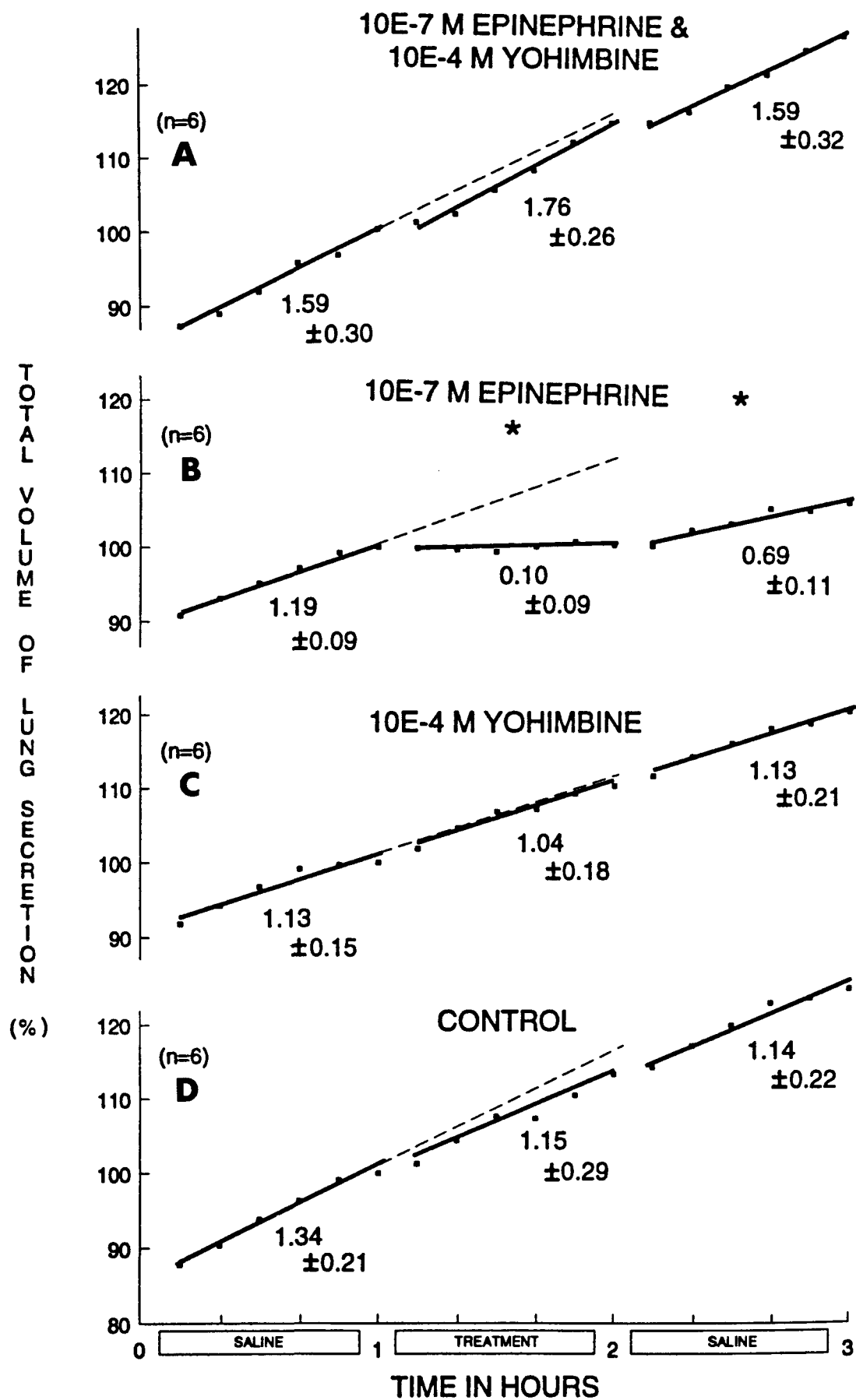
The previous study related to high levels of epinephrine, and it seemed important to extend the effects of the receptor antagonists to more physiological levels, where epinephrine appears to have its maximal effect. Studies were repeated with the two specific alpha receptor antagonists but epinephrine was used at 10^{-7} M.

Epinephrine (10^{-7} M) and yohimbine (10^{-4} M) (Fig. 8)

The studies with the α_2 antagonist yohimbine were repeated at the more physiological level of epinephrine. The results showed that yohimbine at 10^{-4} M blocked the effects of 10^{-7} M epinephrine and the following rates were obtained 1.59 ± 0.30 , 1.76 ± 0.26 , and 1.59 ± 0.32 ml/kg·hr. The fetuses used had an average weight of 77.81 ± 5.08 grams,

Figure 8. The effect of epinephrine (epi) and yohimbine (yoh) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-65 days gestation, with an average body weight of 80.49 ± 2.59 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-7} M epi/ 10^{-4} M yoh (n=6); (B) 10^{-7} M epi (n=6); (C) 10^{-4} M yoh (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



average age of 61 ± 1 days gestation, and an $n=6$. Two way ANOVA shows no significant differences between the rates, although, there was a slight rise in secretion rate in the second hour, perhaps reflecting the marked changes seen in the earlier experiments. 10^{-7} M epinephrine alone caused a significant decrease in secretion rate (Fig. 8B).

Yohimbine (10^{-4} M) (Fig. 8C)

Lungs taken from fetuses ($n=6$, average weight= 78.31 ± 4.64 g, average age of 61 ± 1 days) treated with yohimbine showed rates of 1.13 ± 0.15 , 1.04 ± 0.18 , and 1.13 ± 0.21 ml/kg·hr. No differences between the rates were detected by two way ANOVA.

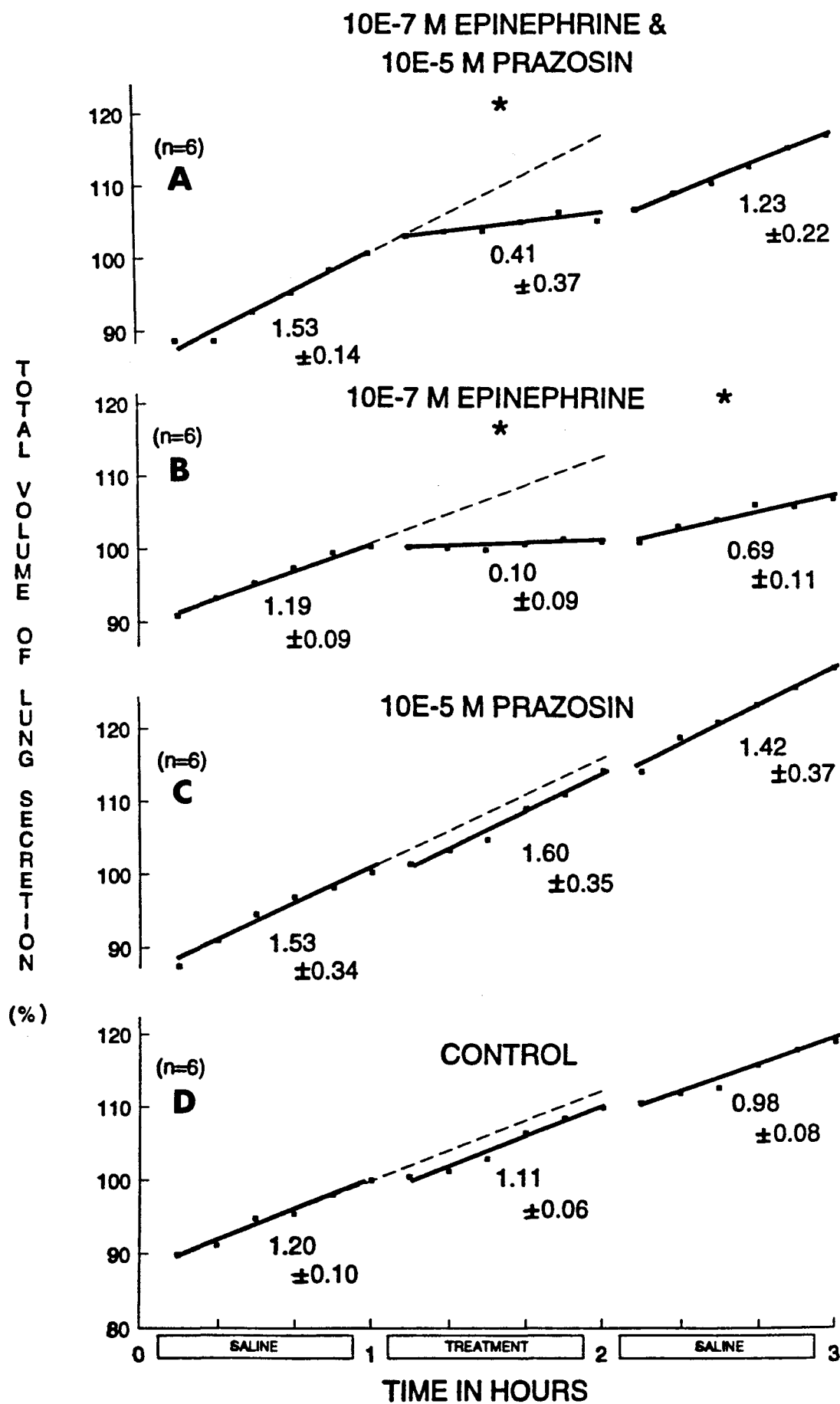
Controls (Figure 8D)

Control experiments were carried out on lungs of fetuses ($n=6$, average weight= 77.59 ± 3.60 g, average age of 63 ± 1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.34 ± 0.21 , 1.15 ± 0.29 , and 1.14 ± 0.22 ml/kg·hr.

The results appear to show that epinephrine at physiological levels works via α_2 receptors to produce a

Figure 9. The effect of epinephrine (epi) and prazosin (pra) on fluid secretion by *in vitro* lungs of fetal guinea pigs.

The data was collected from 24 fetuses, 59-65 days gestation, with an average body weight of 79.37 ± 4.56 (SE) g. The lungs were exposed to the following concentrations after transfer to second hour saline: (A) 10^{-7} M epi/ 10^{-5} M pra (n=6); (B) 10^{-7} M epi (n=6); (C) 10^{-5} M pra (n=6); (D) control (n=6). The slopes represent the secretion rates and the values below the lines are averaged rates in ml/kg·h \pm SE. The asterisks above the lines indicate significant differences from the first hour rate.



significant fall in fluid production; this is in agreement with earlier results.

Epinephrine (10^{-7} M) and prazosin (10^{-5} M) (Fig. 9)

Prazosin at 10^{-5} M could not block the actions of 10^{-7} M epinephrine and the following rates were obtained 1.53 ± 0.14 , 0.41 ± 0.37 , and 1.23 ± 0.22 ml/kg·hr. Two way ANOVA shows significant differences between the rates (Fig. 9A). The lungs used were taken from fetuses with an average weight of 84.01 ± 10.32 grams, average age of 61 ± 1 days gestation, and an $n=6$. The lung fluid production decreased significantly in the second hour when exposed to 10^{-7} M epinephrine alone (Fig. 9B); there was little difference between lungs with and without the antagonist present.

Prazosin (10^{-5} M) (Fig. 9C)

During studies of prazosin alone, rates in successive hours were 1.53 ± 0.34 , 1.60 ± 0.35 , and 1.42 ± 0.37 ml/kg·hr from the lungs of fetuses ($n=6$, average weight= 78.00 ± 4.01 g, average age of 60 ± 1 days). No differences between the rates were detected by two way ANOVA.

Controls (Figure 9D)

Control experiments were carried out on lungs of fetuses (n=6, average weight=67.23±4.74 g, average age of 60±1 days) exposed to Krebs' saline without any drugs throughout the three hours. Two way ANOVA showed no significant difference among the rates: 1.20±0.10, 1.11±0.06, and 0.98±0.08 ml/kg·hr.

The results suggest that stimulation of α_2 receptors, in the absence of functioning α_1 receptors, does not enhance the effects of epinephrine at physiological levels.

General conclusions to studies at physiological levels of epinephrine

Experiments at physiological levels of epinephrine with the specific alpha receptor antagonists showed some similarity to earlier studies at pharmacological concentrations. α_2 -receptors appear to stimulate reabsorption or inhibit secretion of lung fluid; however, the lack of an effect of prazosin at physiological levels of epinephrine suggests that α_1 receptors function only at pharmacological concentrations (Fig. 9).

DISCUSSION

Although research has been done on fetal sheep and goats, the guinea pig is seldom used. The guinea pig may be a better model for human lung development (Stith and Das, 1982) and human reproductive physiology (Martensson, 1984) because both share similar characteristics. Functional development and maturity of the lung (Collins et al., 1986) as well as the development of surfactant are very comparable (Sosenko and Frank, 1987). Also, the regulation of gluconeogenesis are alike in the guinea pig and human (Bergman, 1973). The rates for lung liquid secretion in intact fetal guinea pigs range from 0.2 to 6.7 ml/kg·hr (Setnikar et al., 1959), while the fetal goats have been found to have rates ranging from 1.81 to 12.0 ml/kg·hr (Cassin and Perks, 1982, 1986) and fetal sheep have recorded rates of 1.5 ml/kg·hr (Scarpelli et al., 1975). Our *in vitro* fetal guinea pig lung preparations have produced similar rates with an overall average of 2.14 ± 1.71 (SD) ml/kg·hr (n=450).

The *in vitro* preparations provide a viable fetal lung for 3 hours because the rates from control experiments are similar to those in intact guinea pigs (Setnikar et al., 1959), and they continue to secrete throughout the experiment with no significant change. There is no evidence for cellular damage during incubation because there was no increase in the entry of intracellular potassium or lactic

dehydrogenase into the lung fluid, and the production of lactate was also steady and not unusually high (Perks et al., 1990). In addition, the lungs continued to respond to hormones such as AVP or epinephrine (Perks et al., 1990; Perks et al., 1993).

Epinephrine was discovered in 1895 as a component of adrenal medullary extracts possessing a vasopressor effect (Oliver and Schafer, 1895). The first clue that catecholamines were important in the process of birth came in the late 1960's from studies by Robert S. Comline and Marian Silver of the University of Cambridge. They found that the adrenal glands from fetal cows, horses and sheep produced catecholamines in response to hypoxia without the stimulation of the sympathetic nervous system. In the adult, catecholamines are released only after stimulation of the sympathetic nervous system and its splanchnic nerves, which arise from the central nervous system (CNS). A perceived threat causes the CNS to send impulses to the sympathetic system to release norepinephrine at the target tissue to initiate a "fight or flight" response. Also, the splanchnic nerves through their neurotransmitter acetylcholine stimulate the adrenal medulla to secrete epinephrine into the blood. The "fight or flight" response includes acceleration of heart rate, increase in its output, increase in cardiac muscle contraction, and selective relaxation or constriction of blood vessels to divert blood

away from non-essential organs (skin, intestines, kidneys) to essential organs (heart, brain, skeletal muscles).

The Cambridge findings have led to research into the production and mechanism of catecholamine secretion in the fetus. The adrenal medulla has been found to be directly activated by stress to release fetal catecholamines in the absence of a competent sympathetic nervous system. Slotkin and Seidler (1988) have shown that the development of the nerves to the adrenal glands results in the loss of the gland's ability to respond directly to stress. In rats, the sympathetic system develops rapidly after birth and the adrenal medulla can respond directly to stress only until a few days after birth (Lagercrantz and Slotkin, 1986). The exact timing of full sympathetic maturation in the human is unknown; however, the human adrenal gland is thought to be able to respond directly to hypoxia at birth (Lagercrantz and Slotkin, 1986). Therefore, stress is a very important stimulus to a catecholamine surge during fetal development, and the surge has been shown to be a very effective form of protection. Most stress occurs during the process of birth and to some degree, hypoxia plays a role. Also, animal studies have shown that pressure on the head during uterine contractions can stimulate the sympathetic nervous system to release catecholamines (Lagercrantz and Slotkin, 1986).

Catecholamine levels have been reported to increase approximately 100 fold during delivery (Brown et al., 1980). In the human neonate, levels ranging from 2.31×10^{-8} M

(Faxelius et al., 1984) to 5.0×10^{-8} M (Langercrantz and Slotkin, 1986) have been detected during labour while levels of 6.7×10^{-7} M have been found in the fetal guinea pig (Jelinek and Jensen, 1991). Catecholamine surges play an important role in lung fluid clearance at birth (Walters and Olver, 1978), and also in other changes such as: changes in physiological characteristics to promote normal breathing, including increased cardiac performance particularly during hypoxemia (Lewis et al., 1982), mobilizing glucose and free fatty acids to nourish cells (Comline and Silver, 1972) and pulmonary surfactant release (Lawson et al., 1978).

Woods' results (1990) and Figure 3 show that 10^{-8} M and 10^{-7} M epinephrine caused the greatest reductions in secretion in the fetal guinea pig lung. These levels were comparable to those measured during delivery in both the human and guinea pig. Epinephrine can decrease lung fluid production or cause reabsorption in late term fetal lambs (Walter and Olver, 1978). The lung can also recover to pretreatment fluid production after cessation of epinephrine infusion (Walters and Olver, 1978). Epinephrine also reduced fluid production or stimulated reabsorption in the fetal goat (Perks and Cassin, 1982, 1989).

Figure 3 also shows that 10^{-5} M and 10^{-6} M epinephrine caused no significant change in fluid production rates throughout our experiments. Therefore, optimal effects were obtained at 10^{-7} M and 10^{-8} M epinephrine since at 10^{-9} M, epinephrine had no significant effect on the fetal guinea

pig lung (Woods, 1990). These were unusual observations, because we expected a dose-dependent response throughout the range of epinephrine concentrations used. Woods (1990) found that epinephrine appeared to work through alpha-adrenoreceptors rather than beta-adrenoreceptors in the fetal guinea pig. The work by Woods (1990) with the general alpha and beta adrenergic receptor antagonists, phentolamine and propranolol respectively, was repeated and confirmed in the studies reported here.

Propranolol (beta-adrenoreceptor blocker) appears to have no effect on the actions of epinephrine (Fig. 4) and propranolol did not affect rates when tested alone (Fig.4c). This suggests that epinephrine does not work through beta-adrenoreceptors in the fetal guinea pig lung. However, Walters and Olver (1978) showed that infusions of epinephrine caused reabsorption of fetal sheep lung fluid and propranolol was capable of blocking the effects of epinephrine. They have suggested that the reabsorption of lung fluid at birth is to some degree a result of epinephrine acting on beta-adrenoreceptors. Although Figure 4 disagrees with their findings, one must consider that the experiments were carried out on different species of animals. Beta-adrenoreceptor blockers did not change lung fluid production in the fetal rabbit, at or near birth (McDonald et al., 1986) as well as in fetal lamb (Higuchi et al., 1987, Chapman et al., 1990). Richardson (1979) found that innervation of the lung is species specific and

pulmonary smooth muscle stimulation may be similar in human, guinea pigs and sheep; however, the innervation of the pulmonary epithelium is uncertain and could vary depending on the species. Although beta-adrenoreceptors have been shown to be present in the fetal guinea pig lung, they may exist in the smooth muscle or airway epithelium and not play a significant role in lung fluid movement.

The reabsorptive mechanism in the fetal sheep lung has been worked on for many years and a model based on rectal shark glands has been developed to explain some of the processes (Figure 1) (Gatzky, 1983). In the fetal sheep, epinephrine stimulates beta-adrenoreceptors to increase cAMP inside the cell which in turn opens Na^+ channels in the apical membrane (Walters et al., 1990); however, the precise rate limiting step between the production of cAMP and the opening of the sodium channel is uncertain. Possibly a protein kinase is involved (Walters et al., 1990). The guinea pig lung may have the same mechanisms with which it secretes or reabsorbs lung fluid but the receptors involved appear to be different.

Phentolamine and related agents are widely used general alpha-adrenoreceptor blockers and Figure 5 shows that 10^{-6} M phentolamine blocked the decrease in fluid secretion caused by 10^{-7} M epinephrine. Phentolamine totally eliminated the action of epinephrine; this suggests that epinephrine works through alpha-adrenoreceptors in the guinea pig to cause inhibition of secretion or initiate reabsorption of lung

liquid. Most research on the mechanism of lung fluid secretion and reabsorption has dealt with beta-adrenoreceptors so the discovery of epinephrine acting through alpha-adrenoreceptors was exciting.

Alpha-adrenoreceptors have been found to exist in the fetal guinea pig lung using ^3H -prazosin as a radioligand (Barnes et al., 1979). Experiments using alpha-adrenoreceptor antagonists (Takayanagi et al., 1990) have also shown the presence of alpha-adrenoreceptors in the guinea pig. After confirmation of catecholamines working through alpha adrenoreceptors in the fetal guinea pig lung, we analyzed the unusual observation that epinephrine at high levels (10^{-5} M) failed to affect fluid production. The work used specific alpha-adrenergic receptor antagonists.

The selective antagonism of pressor responses to epinephrine by ergot alkaloids (Dale, 1906) led to the concept of dual, stimulatory and inhibitory catecholamine receptors. This concept was further developed by Alquist (1948) who first classified adrenergic receptors based on pharmacological specificity rather than response type. Ahlquist's method has stood the test of time and has paved the road for many important discoveries such as propranolol, a therapeutic drug used in hypertension, coronary disease and other disorders.

There has been research on the molecular biology, biochemistry and classification of alpha-receptors. For many years, alpha-receptors blockers have been found to

increase the release of norepinephrine produced by the sympathetic nervous system. Starke et al. (1971) and Langer et al. (1971) postulated that the antagonists blocked prejunctional alpha-receptors. Studies with alpha-receptors agonists have shown that the release of norepinephrine was associated with the stimulation of prejunctional alpha-receptors in a negative feedback loop (Starke, 1972). Brown and Gillespie (1957) suggested norepinephrine worked through postjunctional alpha-receptors. The antagonist prevented norepinephrine from binding to the effector cells, thereby causing an increase in norepinephrine release. Further studies led to antagonists and agonists which could distinguish between pre- and postjunctional receptors in vitro. Langer (1974) proposed that the postjunctional receptors mediating effector cells be termed α_1 , while the prejunctional receptors that regulates neurotransmitter release be called α_2 . Support for the differentiation of α_1 and α_2 came from Drew (1976) who provided evidence for pharmacological differences between pre- and postjunctional α -receptors.

Although the anatomical subclassification of alpha-receptors holds true in many cases, a different method of classification had to be used because inhibition of melanin granule dispersion induced by alpha-melanocyte-stimulating hormone in frog skin (Pettinger, 1977) and the inhibition of isoproterenol-induced glycolysis and lipolysis in hamster isolated epididymal adipocytes (Schimmel, 1976) are both

postjunctional events produced by highly selective α_2 agonists. Therefore, the receptors were categorized by functional differences: α_1 -receptors as excitatory and α_2 -receptors as inhibitory. However, there were exceptions to this rule as well, and a new method which is now universally accepted has been developed. The accepted practice for receptor subclassification of alpha-adrenoreceptors is a pharmacological approach based upon highly selective antagonists and to some extent, agonists. The agents used are shown in Table 1.

Table 1. Alpha-adrenoreceptor antagonists and agonists

<u>α_1-adrenoreceptor</u>	<u>Non-selective</u>	<u>α_2-adrenoreceptor</u>
Antagonists:		
prazosin	phentolamine	yohimbine
doxazosin	tolazoline	rauwolscine
Agonists:		
phenylephrine	epinephrine	clonidine
methoxamine	norepinephrine	α -methylnorepinephrine
amidephrine		

Yohimbine, a specific α_2 -adrenoreceptor blocker, at 10^{-4} M caused a significant increase in secretion rate in the second hour when tested with 10^{-5} M epinephrine (Fig.6). This suggests that α_1 -adrenoreceptor stimulation by epinephrine causes the lung to increase production of lung fluid. An increase in liquid secretion has never been seen on a consistent basis until the studies presented here.

Prazosin (10^{-5} M), a specific α_1 -adrenoreceptor antagonist, caused a significant fall in secretion rate when tested with 10^{-5} M epinephrine (Fig.7). This indicates that epinephrine stimulates α_2 -adrenoreceptors to inhibit secretion or stimulate reabsorption of lung fluid. Similar observations have been made on the gastrointestinal tract of different animals where α_2 -adrenoreceptor stimulation with specific agonists induces absorption of sodium and chloride (Durbin et al., 1982 and Chang et al., 1982).

Although experiments with pharmacological levels of epinephrine and alpha-adrenergic receptor antagonists provide good insights into the existence of underlying mechanisms, epinephrine with alpha-adrenoreceptor blockers at physiological levels were also tested to see if similar results could be obtained in a more normal situation.

10^{-4} M yohimbine, the α_2 -receptor antagonist, inhibited the actions of epinephrine at the physiological level (10^{-7} M) (Fig.8). This suggests that, in the guinea pig unlike the sheep, epinephrine reduces fluid production through α_2 -receptors during the normal conditions of birth.

In contrast, prazosin, the α_1 -receptor antagonist which had allowed reabsorption at high levels of epinephrine, had no effect at physiological concentrations of the hormone (Fig. 9). It might have been expected to increase reabsorption at the lower levels of epinephrine;

however, its lack of effect suggests that the α_1 -receptors, which are activated in pharmacological conditions, are simply not active in the normal conditions of birth. Reabsorption of fluid depends on the α_2 -receptor alone.

Results obtained from yohimbine and prazosin present a clear picture of epinephrine working through opposing adrenoreceptors. α_1 -adrenoreceptors appears capable of stimulating secretion while α_2 -adrenoreceptors inhibits secretion or causes reabsorption. Work by Woods (1990) and Figure 3 show that the fetal lung does have a dose dependent epinephrine response which tapers off and stops at high concentrations. Although the actual mechanisms are undetermined, one possibility may be that the lung has α_2 -adrenoreceptors which are more sensitive to epinephrine than α_1 -adrenoreceptors. At low or physiological levels of epinephrine, only the α_2 -adrenoreceptors are stimulated to cause a fall in secretion rate while at high concentrations, both types of alpha-adrenoreceptor are stimulated. The sensitivity of the receptors may be a result of α_2 -adrenoreceptors existing in greater numbers than α_1 -adrenoreceptors; therefore, at low concentrations α_2 -adrenoreceptors are stimulated while α_1 -adrenoreceptors are not. At high concentrations, epinephrine can finally stimulate the few α_1 -adrenoreceptors which exist, as well as the α_2 -adrenoreceptors. When stimulated, the two receptors have opposing mechanisms to eliminate the fall

in secretion rate caused by epinephrine. Also, the α_1 -adrenoreceptor must be a much more dominant receptor when stimulated at pharmacological levels to account for the large difference in numbers between the two receptor types. Takayanagi et al. (1990) have found that α_2 -adrenoreceptors are the predominant subtype in the guinea pig trachea. α_1 receptors have been shown to have a five fold more favourable occupancy response relationship than α_2 receptors in the vascular system of rats (Ruffolo et al., 1979). Also, α_1 receptors have managed to produce half maximal responses at approximately 5 to 10% occupancy in large blood vessels such as the rat aorta (Ruffolo et al., 1979), guinea pig aorta (Ruffolo and Waddell, 1982), canine aorta (Sastre et al., 1984) and rabbit aorta (Besse and Furchgott, 1976; Purdy and Stupechy, 1984). Therefore, at physiological levels of 10^{-7} M epinephrine, lung fluid is reabsorbed or secretion is inhibited while at 10^{-5} M, the α_1 -adrenoreceptors can now respond to epinephrine and oppose the actions of α_2 -adrenoreceptors to keep the second hour secretion rate constant. Only α_2 -adrenoreceptors are working at 10^{-7} M to cause a decrease in secretion, however at 10^{-5} M epinephrine, α_1 -adrenoreceptors and α_2 -adrenoreceptors are both working in opposition to each other.

The alpha-adrenoreceptors and beta-adrenoreceptors are part of a gene family of G protein coupled receptors that

also contain muscarinic receptors, the serotonin 1A adrenoreceptors, and the light sensitive protein, rhodopsin. The genes for the alpha-adrenoreceptors and beta-adrenoreceptors have been sequenced and show a high degree of homology as well as similar topographical organization in the cell membrane. The proteins have seven hydrophobic membrane-spanning α_1 -helical domains which are highly conserved and show the highest degree of sequence homology among the different receptors. The receptor has a N-terminus segment and 3 loops connecting the hydrophobic regions II-III, IV-V, and VI-VII in the extracellular space while the cytoplasmic side contains the C-terminal and 3 additional loops connecting the I-II, III-IV, and V-VI domains (Figure 10). Manipulation of the receptor genes such as point and deletion mutations (Strader et al., 1987 and Chung et al., 1988) as well as construction of chimeric receptors (Kobilka et al., 1988) containing different parts of different receptors have provided structure/activity relationships. Receptor protein proteolysis has also been used to study structure and functional relationship (Dohlman et al., 1987 and Wong et al., 1988). From these studies, tentative conclusions have been made:

- 1) The hydrophobic alpha helices may form a ligand binding protein with one (Lefkowitz and Caron, 1988) or more (Wong et al., 1988) potential binding sites identified with affinity probes. Chimeric α_2/β_2 receptor work has suggested that different structural elements in the hydrophobic core

are responsible for agonist versus antagonist binding (Kobilka et al. , 1988) .

2) Specific amino acid residues such as aspartate 79, 113, 13, and 318 contained in the hydrophobic domain have been found to be important in differentially influencing agonist and antagonist affinities (Strader et al., 1987 and Chung et al., 1988) .

3) The cytoplasmic loops and the carboxyl terminal have been proposed to and shown to couple the receptor to the G proteins (Kubo et al., 1988, and Cotecchia et al., 1988) but there are also conflicting results (Lefkowitz and Caron, 1988) .

4) Activation sites for adenylate cyclase (Wong et al., 1990) as well as activation sites for phospholipase C (Wess et al., 1989) have been found to be located in the third cytoplasmic loop.

The alpha-adrenoreceptors are linked to specific effector systems through intermediary signal-transducing proteins called guanine nucleotide regulatory proteins (G proteins) because they bind and hydrolyze the guanine nucleotide guanosine triphosphate (GTP) (Gilman, 1987) . Alpha₂-adrenoreceptors stimulation with agonists leads primarily to the inhibition of adenylyl cyclase via a distinct G protein, G_i (Bylund, 1988) . In contrast, alpha₁-adrenoreceptors activation leads to the generation of second messengers diacylglycerol and inositol triphosphate by stimulating phospholipase C (Cotecchia et al., 1990) . This

pathway is mediated by a pertussis toxin-insensitive G protein. The beta-adrenoreceptors upon agonist activation stimulates adenylyl cyclase and leads to increased cAMP levels. Mediation of this pathway is through a G protein, G_s . The second messengers, resulting from the activation of G protein coupled receptors, can activate a cascade of events leading to, for example, (1) the activation of specific kinases and subsequent phosphorylation of proteins (Sibley et al., 1987) such as the calcium phospholipid-dependent protein kinase C (PKC) (Taylor et al., 1988, and Blackshear, 1988), (2) release of intracellular calcium stores (Berridge and Irvine, 1984), (3) activation of ion channels (Yatani et al., 1987a, 1987b) or pumps (Michel et al., 1989), (4) release of arachidonic acid (Burch et al., 1986) and (5) gene transcription. However, recent data which suggests individual receptor subtypes may be capable of coupling to different G proteins (Cotecchia et al., 1990, Peralta et al., 1988, and Fargin et al., 1989) further illustrates the complexity of receptor activated signalling networks.

Alpha₁-adrenoreceptors activation produces changes in cellular activity by increasing intracellular levels of free Ca^{2+} . This is accomplished by coupling of a G protein with phospholipase C to initiate hydrolysis of a membrane phospholipid phosphatidylinositol bisphosphate, to produce two second messengers: (1) diacylglycerol (DAG) which activates PKC, and (2) inositol 1,4,5-trisphosphate (IP₃)

which acts to release calcium stored intracellularly (Berridge and Irvine, 1989). The α_1 -adrenoreceptors are also coupled directly to a receptor-operated Ca^{2+} channel to increase calcium influx (Han et al., 1987).

α_2 -adrenoreceptors are coupled by G_i protein to adenylate cyclase or alternatively to ion channels. Thus, cellular activity is altered either by reducing intracellular levels of cAMP or by directly modifying the activity of ion channels such as the Na^+/H^+ antiport, Ca^{2+} channels, or K^+ channels (Bylund, 1988).

The results from yohimbine and prazosin (Figures 6,7,8 and 9) show the specific receptors through which epinephrine works; however, the receptor mediated change in cellular activity could not be determined. α_1 -adrenoreceptors appear to carry out their function by increasing intracellular calcium levels which then lead to change in cellular activity. The actual cascade of events leading to the stimulation of lung fluid secretion, however, is not known.

The α_2 -adrenoreceptors regulation, on the other hand, is much more difficult to explain for the fetal lung. Although the α_2 -adrenoreceptors have been implicated to inhibit adenylyl cyclase and decrease cAMP levels, the fetal guinea pig as well as fetal sheep has been found to reabsorb lung fluid when cAMP levels rise (Walters et al., 1990 and Kindler et al., 1992). Therefore, the α_2 -adrenoreceptor may be coupled to an ion channel, possibly Ca^{2+} which can

act as a second messenger or perhaps the α_2 -receptor behaves differently in the fetal lung to increase rather than decrease cAMP to cause reabsorption of lung fluid. Once again, however, the actual events are not known.

These studies on the effects of epinephrine on alpha-adrenoreceptors of fetal guinea pig lung fluid provide some insights into the mechanism of liquid secretion and reabsorption. The α_1 -adrenoreceptors and α_2 -adrenoreceptors appear to work in opposition where α_1 -adrenoreceptor stimulate and α_2 -adrenoreceptor inhibit, which has been one method of classification of alpha-adrenoreceptors. Also, the α_2 -adrenoreceptors have been found to induce absorption of fluid in the gastrointestinal tract of rabbit and human (Chang et al., 1982, Durbin et al., 1982 and Rubinoff et al., 1989).

The *in vitro* preparations provide good insights into the effects of drugs on the secretion and reabsorption of lung fluid but in order to get a more detailed understanding of the mechanism, molecular techniques such as autoradiography and gene manipulation, must be used. Hopefully, the present study has shed some light on the mechanisms of lung fluid secretion and reabsorption and will help better understand lung diseases such as respiratory distress syndrome, and eventually lead to cures.

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