Effects of glucagon on in vitro liquid production by lungs from fetal guinea pigs

N Choo, A L Liu, A M Perks

Abstract

Background—Lung liquid reabsorption in newborns with respiratory distress syndrome can be deficient. Respiratory distress syndrome is often seen in infants of diabetic mothers, in whom the neonatal surge of glucagon is suppressed.

Aim—To investigate the possible effects of glucagon on lung liquid reabsorption.

Methods—Lungs from near term fetal guinea pigs (62 (2) days gestation; term = 67 days) were supported in vitro for three hours; lung liquid production and reabsorption were monitored by a dye dilution method.

Results—Untreated control preparations produced fluid at 1.75 (0.33) ml/h per kg body weight, and did not change significantly in three hours; those immersed in 10^{-12} M glucagon during the middle hour showed no significant change, but those given higher concentrations all showed significant reductions in fluid production or even reabsorption (65.6 (10.3)% fall at 10^{-11} M, 70.0 (6.3)% fall at 10^{-10} M, and 90.6 (11.1)% fall at 10^{-9} M; based on 54 preparations). At 10^{-9} M glucagon, 12 out of 30 preparations reabsorbed fluid. The linear log dose-response curve ($r^2 = 0.94$) gave a theoretical threshold at 4×10^{-15} M glucagon. Responses appeared to involve the amiloride sensitive Na^+ based reabsorptive system: responses to 10^{-9} M glucagon appeared to be reduced by 10^{-6} M amiloride, and were abolished by 10^{-5} M amiloride (based on 72 preparations).

Conclusions—The results suggest that the surge of glucagon at birth may help to drain the lungs of fluid. As glucagon liberates cAMP, which also stimulates surfactant, glucagon is worth consideration for possible use in neonatal respiratory distress.

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Keywords: glucagon; fetus; lung liquid; guinea pig; respiratory distress syndrome

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It is vital to clear the lungs of fluid at the time of birth. Up to that time, fluid has been actively secreted by a Cl^- based mechanism ($Na^+/K^+/2Cl^-$ cotransport), probably present in pulmonary type II cells.[1] At birth, this is replaced by a Na^+ based reabsorptive system, at least partly dependent on amiloride sensitive Na^+ channels present in the apical membranes of type II cells.[1 2] The mechanisms that produce this change are probably numerous, as the process is vital to the newborn, and each influence forms an insurance for failure of the others.

Despite this, failures do occur; they are seen in situations related to hypoxia,[2] prematurity, and caesarean section,[3] and, most significantly, in respiratory distress syndrome, a major cause of newborn death.[4]

The factors that can reverse fluid movement at birth fall into four main categories: hormonal (adrenaline,[1] arginine vasopressin (AVP),[5] adrenal steroids[6]); physical (expansion of the lungs,[7] fall in temperature,[8]); paracrine (products of the neuroendocrine system of the lungs[9–11]); neural (acetyl choline,[12] noradrenaline[13]). Although a variety of second messengers may be involved, at least two of the hormones are known to act through the adenylate cyclase system (adrenaline and β-agonists,[14] AVP[15]), and cAMP, its analogues, and forskolin are known to produce fluid reabsorption.[1 16] Therefore it seemed reasonable that other activators of adenylate cyclase may also produce fluid reabsorption.

Glucagon has held a special place in our understanding of the adenylate cyclase system. It was studies of its mode of action that first uncovered cAMP, and it is known to be a powerful activator of this system.[17 18] It is also known to surge to high plasma levels immediately after birth in every species tested, including man (rat,[19] rabbit,[20] sheep,[21] human[22]). In addition, glucagon receptors are found in lungs, surprising for a hormone mainly associated with carbohydrate metabolism in the liver, and transcripts of their mRNA are found in unusually high levels in lungs of fetal and young animals.[23 24] Although the functions of these receptors are not known, Campos *et al*[24] suggested, perhaps with notable insight, that they may be concerned with functions in the fetal lung, rather than actions in the adult.[24] Clearly, there were many indications that glucagon may be involved in drainage of the newborn lung, but no studies were ever made.

In the work presented here, glucagon is shown to be capable of activating fluid reabsorption in vitro by lungs from fetal guinea pigs, a new observation that may have useful consequences.

Materials and methods

ANIMALS

Pregnant albino guinea pigs of an inbred departmental stock were given food and water ad libitum (guinea pig chow, Ralston-Purina, supplemented with fresh vegetables and vitamin C). Treatment of the animals was in accordance with the Canadian Council for Animal Care, and in conditions approved by the animal care committee of the University of British Columbia. Studies were performed on

fetuses of 62 (2) days gestation (term = 67 days) and 89.6 (18.9) g body weight (means (SD)).

EXPERIMENTAL PROCEDURES
Methods for measuring rates of lung liquid production and reabsorption have been detailed and confirmed for validity in previous publications.[12 25 26] In brief, lung liquid production or reabsorption was measured by an impermeant tracer technique based on Blue Dextran 2000 (Pharmacia, Dorval, Que, Canada). Pregnant guinea pigs were anaesthetised with halothane, and the fetuses removed by caesarean section. The trachea of each fetus was cannulated towards the lungs with polyethylene tubing filled with saline and connected to a 1.0 ml tuberculin reservoir syringe. The lungs were removed and supported in baths of Krebs-Henseleit saline at 37°C bubbled with 95% O_2 and 5% CO_2. Then 100 μl Blue Dextran 2000 (50 mg/ml in 0.9% NaCl) was mixed into the lung liquid, and the preparations were equilibrated for 30 minutes. During the experimental period (three hours), lung liquid was withdrawn into the reservoir syringe every 10 minutes, and 10 μl samples were removed with a 1701 NCH gas tight fixed volume syringe (Hamilton Co, Reno, Nevada, USA). These samples were diluted 1:20 with distilled water, vortex mixed, and centrifuged for 10 minutes at 250 g; Blue Dextran in the supernatants was estimated by spectrophotometry ($λ = 620$ nm). Throughout the experiment, proper mixing of dye within the lungs was ensured by withdrawing and returning 0.3 ml of lung liquid into the reservoir syringe every five minutes and by the gentle but continuous movements of the lungs in the bubbled saline. The experiments followed an ABA design. Samples taken during the first hour after equilibration gave the resting rate of fluid production. The lungs, still attached to their reservoir syringe, were then transferred to fresh Krebs-Henseleit saline which contained one of the following: (*a*) synthetic glucagon at 10^{-12}, 10^{-11}, 10^{-10}, or 10^{-9} M; (*b*) 10^{-9} M glucagon with 10^{-6} M amiloride hydrochloride (amiloride was placed in both the outer saline (basally) and the lung fluid (apically)); (*c*) 10^{-9} M glucagon with 10^{-5} M amiloride hydrochloride used in the same way; (*d*) 10^{-6} or 10^{-5} M amiloride hydrochloride alone, used as before; (*e*) Krebs-Henseleit saline with no drugs (untreated controls (these controls received changes of saline on each hour, as for experimental lungs). Amiloride was placed both inside and outside the preparations in order to remove the concentration gradient, which would have caused loss from the apical side of the epithelium. All reagents were from Sigma (St Louis, MO, USA).

In all experiments, the preparations were transferred back to Krebs-Henseleit saline for the final hour.

QUANTIFICATION OF RESULTS AND STATISTICAL METHODS
The rates of fluid production were calculated from the fall in concentration of Blue Dextran, as described previously.[25 26] Rates were estimated from plots of the total volume of fluid against time, with readings recorded every 10 minutes; the total volume of fluid was the sum of that within the lungs and that removed for study. Appropriate sequential adjustments were made every 10 minutes for removal of both fluid and Blue Dextran during incubation. The rates of production of fluid over one hour intervals were calculated from the volume plots, using the slopes of their regressions, fitted by the method of least squares (Hewlett-Packard program SD-03A, or Apple II Plus computer). In groups of similar experiments, differences in rates in successive hours were analysed by analysis of variance and Newman-Keul's test (ANOVA). When plots from similar experiments were combined, the volumes were expressed as a percentage of the volume present at the end of the first hour, just before transfer to test solutions or fresh control saline; the values were then averaged.[25 26] The significance of changes in rate were also assessed from the combined graphs by submitting the changes in slopes to a test for differences between two regressions (regression analysis)[25 26]; this test used all values for volumes from all experiments in the group. Whereas both ANOVA and regression analysis considered the magnitude of the changes seen, ANOVA took into consideration the repeatability of the responses, and regression analysis allowed for scatter around the lines of best fit, a variability not included in ANOVA. All mean values are given with their standard errors, unless otherwise indicated. Statistical significance was accepted at or below p = 0.05.

Results
EFFECTS OF GLUCAGON ON LUNG LIQUID PRODUCTION IN VITRO
Basic studies were carried out on 54 in vitro preparations of lungs from fetal guinea pigs of 62 (2) days gestation and 90.2 (18.8) g body weight (means (SD)). Initially, 30 preparations were divided into groups of six, but data from those given 10^{-9} M glucagon were supplemented with results from later studies. During the middle hour of the three hour incubations, the preparations received 10^{-12}, 10^{-11}, 10^{-10}, or 10^{-9} M glucagon, or saline alone (untreated controls). Figure 1 shows the results, and table 1 the significance of changes.

Six untreated (control) preparations and six given 10^{-12} M glucagon continued to produce fluid with no significant change (ANOVA; regression analysis) (fig 1A,B respectively). Six lungs given 10^{-11} M glucagon all reduced production, and one showed reabsorption: the average reduction of 65.6 (10.3)% was significant by both statistical tests (fig 1C). Greater and significant reductions were shown by all preparations at higher concentrations of hormone: average reductions were 70.0 (6.3)% at 10^{-10} M glucagon and 90.6 (11.1)% at 10^{-9} M glucagon (fig 1D,E). At the highest concentration, 12 of the 30 preparations turned to reabsorption. Good or partial recoveries were found in all groups after removal of glucagon. Responses quantified as percentage reductions
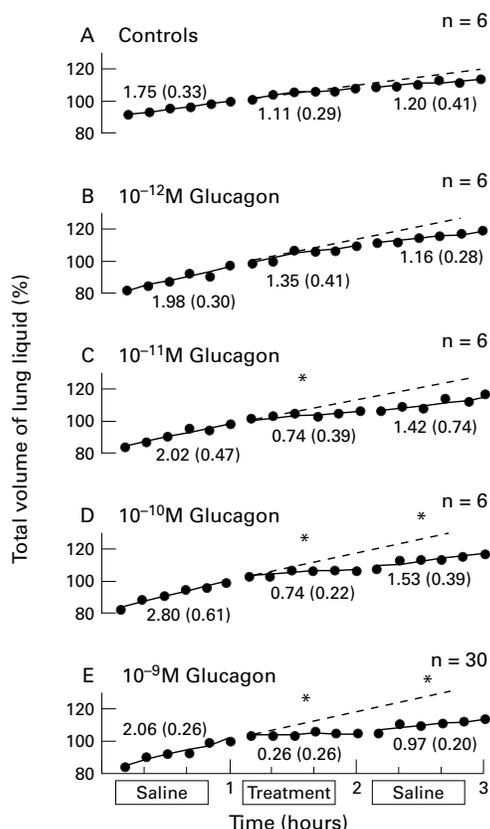
Figure 1 Effects of glucagon on in vitro liquid production by lungs from fetal guinea pigs. Based on 54 fetuses of 62 (2) days gestation and 90.2 (18.8) g body weight. During the middle hour, the preparations were immersed in saline containing one of the following: (A) saline alone (untreated preparations); (B) 10^{-12} M glucagon; (C) 10^{-11} M glucagon; (D) 10^{-10} M glucagon; (E) 10^{-9} M glucagon. Ordinates: total volume of lung liquid expressed as a percentage of that present at the end of the first hour, where 100% was: (A) 1.15 (0.17) ml; (B) 0.75 (0.13) ml; (C) 1.05 (0.49) ml; (D) 1.36 (0.77) ml; (E) 1.04 (0.40) ml; all means (SD). All regressions are lines of best fit; slopes represent production rates. Values below the lines are mean (SEM) production rates in ml/h per kg body weight. Asterisks above the lines show significant changes from the original slope (dashed lines). Standard errors are omitted for clarity, but they averaged: (A) 1.41 (0.18); (B) 2.74 (0.27); (C) 3.67 (0.50); (D) 2.90 (0.22); (E) 2.01 (0.22). Corresponding coefficients of variation were: (A) 1.65 (0.39)%, (B) 9.44 (0.93)%; (C) 8.23 (1.94)%; (D) 6.75 (0.52)%; (E) 10.50 (1.00)%.

during treatment showed a linear relation with log dose ($r^2 = 0.94$), with a theoretical threshold at 4×10^{-15} M glucagon (fig 2). Over the groups studied, there was no relation between response and age or weight; however, in additional tests of four fetuses below 65 g body weight, two failed to respond.
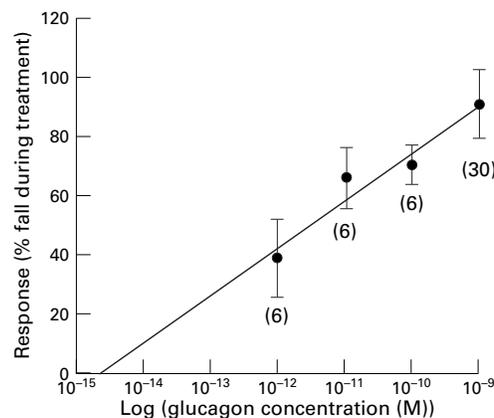

Figure 2 Log dose-response curve for effects of glucagon on in vitro liquid production by lungs from fetal guinea pigs. Based on 48 fetuses of 62 (2) days gestation and 91.8 (18.9) g body weight. Ordinate: responses quantified as percentage falls in production between the hour before and the hour during treatment; error bars represent SEM, and the numbers of fetuses are given below the bars. Abscissa: concentrations of glucagon (M) on a log scale.

EFFECTS OF 10^{-6} M AMILORIDE

Amiloride was used to determine whether glucagon acted through the amiloride sensitive Na^+ based reabsorptive system activated by other agents.

Initial studies were made on 36 preparations of in vitro lungs from fetal guinea pigs of 61 (2) days gestation and 93.1 (17.3) g body weight. They were divided into three groups of 12 lungs: one group received 10^{-9} M glucagon alone, one group received 10^{-9} M glucagon together with 10^{-6} M amiloride, and one group formed the controls (six with 10^{-6} M amiloride alone, and six with saline only (untreated controls)). Figure 3 shows the results, and table 2 the significances of changes.

Amiloride at a concentration of 10^{-6} M reduced, but did not abolish, responses to glucagon. In these studies, 10^{-9} M glucagon caused significant reductions in fluid production in all 12 preparations, with eight turning to reabsorption; the average reduction was large (130.4 (21.1)% fall: fig 3A). When 10^{-9} M glucagon was tested in the presence of 10^{-6} M amiloride, three preparations showed no response; however, six still reduced production, and three turned to reabsorption; the average reduction was still significant, but had halved to 67.5 (18.9)% (fig 3B). Comparison of percentage reductions with and without amiloride showed that the responses were significantly reduced in the presence of the channel blocker (ANOVA: $p < 0.05$). Preparations given 10^{-6} M amiloride alone and untreated

Table 1 Effects glucagon on lung liquid production

| Concentration of glucagon (M)	n	Rate of production of lung liquid (ml/h per kg body weight)			Reductions from the hour before treatment (ml/h per kg body weight)		Significance of reductions during treatment (p value)		Significance of reductions after treatment (P)	
		Hour before treatment	Hour during treatment	Hour after treatment	Hour during treatment	Hour after treatment	ANOVA	Regression analysis	ANOVA	Regression analysis
0 (controls)	6	1.75 (0.33)	1.11 (0.29)	1.20 (0.14)	0.64 (0.29)	0.55 (0.22)	>0.05	>0.05	>0.05	>0.05
10^{-12}	6	1.98 (0.30)	1.35 (0.41)	1.16 (0.28)	0.63 (0.24)	0.82 (0.30)	>0.05	>0.05	>0.05	>0.05
10^{-11}	6	2.02 (0.47)	0.74 (0.39)	1.42 (0.74)	1.28 (0.57)	0.60 (0.41)	<0.05	<0.025	>0.05	>0.05
10^{-10}	6	2.80 (0.61)	0.74 (0.22)	1.53 (0.39)	2.06 (0.48)	1.27 (0.38)	<0.005	<0.01	<0.025	<0.05
10^{-9}	30	2.06 (0.26)	0.26 (0.26)	0.97 (0.20)	1.80 (0.28)	1.09 (0.03)	<0.001	<0.0005	>0.05	<0.01

All values are mean (SEM).
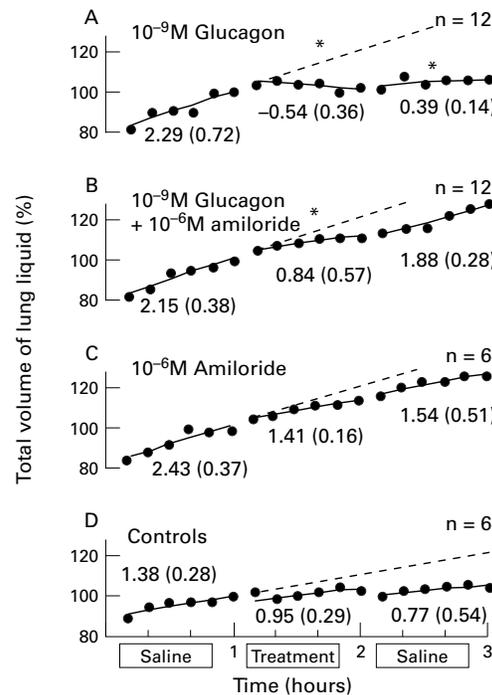
Figure 3 Effect of 10^{-6} M amiloride on in vitro responses to glucagon by lungs from fetal guinea pigs. Based on 36 fetuses of 61 (2) days gestation and 93.1 (17.3) g body weight. During the middle hour, the preparations were immersed in saline containing one of the following: (A) 10^{-9} M glucagon alone; (B) 10^{-9} M glucagon with 10^{-6} M amiloride; (C) 10^{-6} M amiloride alone; (D) saline alone (untreated controls). Ordinates: total volume of lung liquid expressed as a percentage of that present at the end of the first hour, where 100% was: (A) 0.85 (0.22) ml; (B) 0.81 (0.10) ml; (C) 1.20 (0.31) ml, (D) 1.09 (0.24) ml; all means (SD). All regressions are lines of best fit; slopes represent production rates. Values below the lines are mean (SEM) production rates in ml/h per kg body weight. Asterisks above the lines show significant changes from the original slope (dashed lines). Standard errors are omitted for clarity, but they averaged: (A) 2.64 (0.23); (B) 2.58 (0.24); (C) 5.87 (0.62); (D) 2.61 (0.28). Corresponding coefficients of variation were: (A) 9.06 (0.73)%; (B) 8.19 (0.76)%; (C) 12.69 (1.16)%; (D) 6.39 (0.68)%.
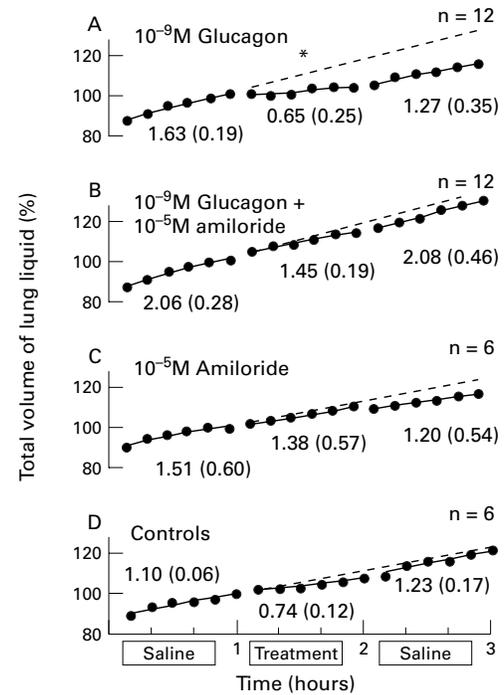
Figure 4 Effect of 10^{-5} M amiloride on in vitro responses to glucagon by lungs from fetal guinea pigs. Based on 36 fetuses of 63 (2) days of gestation and 94.5 (19.7) g body weight. During the middle hour, the preparations were immersed in saline containing one of the following: (A) 10^{-9} M glucagon alone; (B) 10^{-9} M glucagon with 10^{-5} M amiloride; (C) 10^{-5} M amiloride alone; (D) saline alone (untreated controls). Ordinates: total volume of lung liquid expressed as a percentage of that present at the end of the first hour, where 100% was: (A) 1.10 (0.38) ml; (B) 1.25 (0.24) ml; (C) 0.83 (0.18) ml; (D) 0.96 (0.24) ml; all means (SD). All regressions are lines of best fit; slopes represent production rates. Values below the lines are mean (SEM) production rates in ml/h per kg body weight. Asterisks above the lines show significant changes from the original slope (dashed lines). Standard errors are omitted for clarity, but they averaged: (A) 1.37 (0.17); (B) 2.52 (0.29); (C) 1.90 (0.34); (D) 1.74 (0.21). Corresponding coefficients of variation were: (A) 2.19 (0.52)%; (B) 7.87 (0.76)%; (C) 4.28 (0.71)%; (D) 3.96 (0.43)%.

controls showed no significant changes (fig 3C,D).

EFFECTS OF 10^{-5} M AMILORIDE

As only partial reductions were seen with 10^{-6} M amiloride, the concentration was increased to 10^{-5} M. Studies were made on 36 preparations from guinea pigs of 63 (2) days gestation and 94.5 (19.7) g body weight. They were divided into the same groups as used previously. Figure 4 shows the results, and table 2 the significances of changes.

At a concentration of 10^{-5} M, amiloride abolished responses to glucagon. Glucagon at 10^{-9} M produced the usual significant effects, with 10 preparations reducing production and two turning to reabsorption (fig 4A). In the presence of 10^{-5} M amiloride, there was no longer any significant reduction in production (ANOVA; regression analysis; fig 4B). ANOVA also showed that the percentage changes with and without amiloride were significantly different from one another (p < 0.01). Preparations given 10^{-5} M amiloride alone and untreated controls showed no significant changes (fig 4C,D).

It was concluded that the largest responses produced by glucagon could be reduced by

Table 2 Effects of amiloride on responses to glucagon

Concentration of glucagon (M)	Concentration of amiloride (M)	n	Rate of lung liquid production (ml/h per kg body weight)			Reductions from the hour before treatment (ml/h per kg body weight)		Significance of reductions during treatment (p value)		Significance of reductions after treatment (P)	
			Hour before treatment	Hour during treatment	Hour after treatment	Hour during treatment	Hour after treatment	ANOVA	Regression analysis	ANOVA	Regression analysis
10^{-9}	0	24	1.72 (0.14)	0.05 (0.29)	0.79 (0.14)	1.67 (0.25)	0.93 (0.24)	<0.001	<0.0005	>0.05	<0.01
10^{-9}	10^{-6}	12	2.15 (0.38)	0.84 (0.57)	1.88 (0.28)	1.31 (0.39)	0.27 (0.10)	<0.025	<0.0025	>0.05	>0.05
0	10^{-6}	6	2.43 (0.37)	1.41 (0.16)	1.54 (0.51)	1.02 (0.38)	0.89 (0.33)	>0.05	>0.05	>0.05	>0.05
10^{-9}	10^{-5}	12	2.06 (0.28)	1.45 (0.19)	2.08 (0.46)	0.61 (0.14)	−0.02 (0.01)	>0.05	>0.05	>0.05	>0.05
0	10^{-5}	6	1.51 (0.60)	1.38 (0.57)	1.20 (0.54)	0.13 (0.04)	0.31 (0.12)	>0.05	>0.05	>0.05	>0.05
Untreated controls		12	1.59 (0.20)	1.06 (0.17)	0.96 (0.28)	0.53 (0.17)	0.63 (0.21)	>0.05	>0.05	>0.05	>0.05

All values are mean (SEM).

10^{-6} M amiloride and abolished by 10^{-5} M amiloride.

Discussion

The results presented here show that glucagon at concentrations between 10^{-11} M and 10^{-9} M could reduce lung liquid production or produce reabsorption in vitro by lungs from fetal guinea pigs. The responses were linearly related to the log dose ($r^2 = 0.94$), and the greatest number of preparations that turned to reabsorption were seen at the highest concentration. The theoretical threshold indicated by the log dose-response curve was low (4×10^{-15} M), but effects could not be shown to be significant until glucagon reached 10^{-11} M; nevertheless, the preparations appeared to depend on an amiloride sensitive Na^+ based reabsorptive system, as those at the most effective concentration of glucagon tested (10^{-9} M) appeared to be reduced by 10^{-6} M amiloride and were abolished by 10^{-5} M amiloride.

These results suggest a new role for glucagon. An effect in activating lung liquid reabsorption at the time of birth would be reasonable for many reasons. Like other activators of reabsorption, such as adrenaline and AVP, glucagon is a powerful activator of the adenylate cyclase system, and cAMP, its analogues, and forskolin are powerful stimulators of lung fluid reabsorption in fetal sheep and guinea pigs[1 16]; in fact, in our in vitro preparations, forskolin, a direct stimulator of adenylate cyclase, produced the most powerful reabsorptions yet seen.[16] As pointed out already, glucagon was central to the discovery of the adenylate cyclase system,[17 18] and it is hardly surprising that it may promote drainage of the newborn lung; however, no study of this has ever been made.

Despite its clear effects, we must consider whether there is sufficient plasma glucagon to have a physiological effect at the time of birth. In fact, glucagon, like adrenaline and AVP, surges to high plasma levels just after birth. Concentrations in various species lie mainly between 10^{-10} M and 10^{-9} M glucagon: rat, $(2.6\text{--}3.4) \times 10^{-10}$ M[19 27]; rabbit, 1.78×10^{-10} M[20]; sheep, 0.68×10^{-10} M[21]; human, $(0.4\text{--}4.6) \times 10^{-10}$ M[22 27--29]. The clearest data, those for sheep, show a rapid rise in the plasma immediately after cutting of the umbilical cord, and, although levels may halve within an hour in sheep, or within 24 hours in rats, they can remain elevated for many days during suckling.[21 30] Nevertheless, the reduction after the initial surge implies that some plasma levels quoted, especially in humans, may have been too late to catch the true maximum in the blood. Therefore 10^{-10} M plasma glucagon is probably a conservative estimate, and values up to 10^{-9} M are reasonable. In our studies, 10^{-10} M glucagon reduced lung liquid production by 70 (6)%, and 10^{-9} M resulted in a 90 (11)% fall, with 12 out of 30 preparations reabsorbing fluid. Therefore, our results could reflect the true physiological situation.

In addition, responses at birth would be far greater for the following reasons.

(*a*) It is well known that responses involving lung liquid and the adenylate cyclase system mature and increase considerably near birth. This has been seen for responses to adrenaline, AVP, cAMP itself, and to expansion.[1 31--33] Our fetuses were relatively young in such terms; even so, strong reabsorption did occur (fig 3A). There was no evidence for maturational change within our groups, because of the narrow age range chosen, a deliberate selection to allow results to be combined and based on many individuals. The only indication of such changes comes from four notably small fetuses, in which the effect appeared to arise around 65 g body weight. Nevertheless, in another organ, the liver of the fetal guinea pig, increased coupling of glucagon receptors occurs remarkably late, probably only on the day of birth and in the newborn,[34] therefore large responses of the lungs may also occur late, later than the preparations studied here.

(*b*) In vivo, responses would be augmented by colloid osmotic effects, which were absent from our in vitro preparations. This also implies that those in vitro preparations that only stopped production in our experiments could be expected to reabsorb fluid in the intact newborn.

(*c*) In vivo, the effects may form part of a far more complex and effective system, and be augmented through the adrenal medulla. Glucagon has been shown to release catecholamines from adult adrenals.[35 36] In its turn, adrenaline can stimulate fluid reabsorption, and in our in vitro preparations, noradrenaline can act similarly.[13] Therefore, in the intact animal, glucagon may act directly on the lungs, but also indirectly through the adrenal medulla. In our isolated lungs, the indirect effect through the adrenals would not be seen. However, it is also possible that glucagon may release catecholamines within the pulmonary tissue; acetylcholine stimulates fluid reabsorption in this way.[12] These observations raise interesting possibilities: catecholamines can release glucagon in fetuses of rats and sheep, and glucagon can also release catecholamines, so that a powerful self activating cycle may exist.[37 38] However, the concentration of glucagon needed to release catecholamines is usually high, as judged by work in adults.[36] Therefore this positive feedback could only work if the adrenals of neonates were notably sensitive to glucagon.

Glucagon appears to act through the same amiloride sensitive Na^+ based reabsorptive system as activated by adrenaline in fetal sheep, by AVP in fetal sheep and guinea pigs, and by aldosterone in the guinea pig.[1 5 6 32] Our results for glucagon are closely similar to those for aldosterone. Responses produced by the highest concentration of glucagon appeared to be reduced by almost half by 10^{-6} M amiloride, and responses were abolished in some preparations. Although this low concentration of amiloride did not abolish all responses, the overall reduction in their magnitude was significant ($p < 0.05$); therefore the Na^+ channel blocker did have some effect. In contrast, at 10^{-5} M amiloride all responses were abolished, and

ANOVA showed that the difference was significant at p < 0.01. If the comparison were made with all the experiments with 10^{-9} M glucagon from both sets of amiloride studies (n = 24; figs 3A and 4A), the difference becomes highly significant at p < 0.0025. This result was notable; work on fetal sheep has needed amiloride at much greater concentrations to give clear results (at or above 10^{-4} M).[1 5] Therefore the levels used here may be considered relatively conservative. Nevertheless, at concentrations above 10^{-6} M, amiloride starts to have effects beyond simple blockage of sodium channels, effects such as inhibition of Na^+/H^+ exchange, and at 10^{-3} M, it can slow the fundamental Na^+/K^+ ATPase.[39] However, our results for 10^{-6} M amiloride suggest that Na^+ channels are involved, and the total inhibition at 10^{-5} M amiloride shows that the classical Na^+ based transport system is responsible for the fluid reabsorption.

The effects of glucagon may help to explain the failure of β-adrenoreceptor antagonists to prevent fluid reabsorption during natural deliveries: the irreversible β-blocker bromacetylprenolomenthane fails to stop fluid clearance in rabbits either at or after delivery,[40] and propranolol fails similarly during spontaneous labour in chronic fetal sheep.[41] In addition, propranolol cannot prevent slowing of fluid production during moderate asphyxia in fetal sheep, and asphyxia is often a complicating factor during vaginal deliveries.[42] A reabsorptive mechanism independent of β-adrenoreceptors is needed, and, together with effects of lung expansion,[33] glucagon may help to fill this need.

An effect of glucagon on lung liquid may prove a major function. Its traditional role in liberating glucose stores at birth has been brought into question. It requires high levels of glucagon to induce glucose generation by the liver of near term fetal guinea pigs, in which receptor numbers are low and the receptor-adenylate cyclase system is insensitive.[34 43 44] There is similar insensitivity in terms of generation of cAMP in the rat.[34] In contrast, the reactions of the fetal lungs appear to take place at physiological concentrations. Therefore the surge of glucagon at birth may prove more important to the lungs than to its traditional target organ, the liver.

Finally, it may be significant that the surge of glucagon at birth fails in infants of diabetic mothers, and these infants show a high incidence of respiratory distress syndrome.[4 22] Glucagon at high levels is already in clinical use for diagnosing pheochromocytoma[36]; its ability, at far lower levels, to liberate cAMP, which can not only remove lung liquid but also stimulate surfactants,[45] suggests that it may be considered for possible use in problems of the newborn lung. This is a particularly attractive possibility, as glucagon is a natural constituent of the body, not a drug, and its potential side effect of raising blood glucose could be expected to be weak, and probably of little disadvantage.

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