

Role of ATP-Dependent Potassium Channels in Pulmonary Vascular Tone of Fetal Lambs With Congenital Diaphragmatic Hernia

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ABSTRACT: High mortality in newborn babies with congenital diaphragmatic hernia (CDH) is principally due to persistent pulmonary hypertension. ATP-dependent potassium (K_{ATP}) channels might modulate pulmonary vascular tone. We have assessed the effects of Pinacidil, a K_{ATP} channel opener, and glibenclamide (GLI), a K_{ATP} channel blocker, in near full-term lambs with and without CDH. *In vivo*, pulmonary hemodynamics were assessed by means of pressure and blood flow catheters. *In vitro*, we used isolated pulmonary vessels and immunohistochemistry to detect the presence of K_{ATP} channels in pulmonary tissue. *In vivo*, pinacidil (2 mg) significantly reduced pulmonary vascular resistance (PVR) in both controls and CDH animals. GLI (30 mg) significantly increased pulmonary arterial pressure (PAP) and PVR in control animals only. *In vitro*, pinacidil (10 μ M) relaxed, precontracted arteries from lambs with and without CDH. GLI (10⁻⁵ μ M) did not raise the basal tone of vessels. We conclude that activation of K_{ATP} channels could be of interest to reduce pulmonary vascular tone in fetal lambs with CDH, a condition often associated with persistent pulmonary hypertension of the newborn. (*Pediatr Res* 60: 537–542, 2006)

A unique hemodynamic adjustment takes place within 24 h after delivery. Under normal circumstances, pulmonary arterial pressure (PAP) falls by 50% and the concomitant 10-fold increase in pulmonary arterial blood flow (1) is associated with redistribution of blood (1,2). The different factors responsible for this adjustment are the establishment of an air-fluid interface with ventilation (3), an increase of oxygen concentration in the blood (4) and shear-stress (5). Children

born with congenital diaphragmatic hernia (CDH) have two hypoplastic lungs associated with pulmonary hypertension (1) resulting in hypoxemia, acidosis and heart failure.

Postnatal pulmonary vascular tone is influenced by various vasoconstrictors or vasodilators. Vasoconstrictors such as endothelin-1 (ET-1) or thromboxane A₂ increase the vascular resistance (6,7), although ET-1 may also play a role in vasodilation in the new-born (8). There exist direct vasodilators such as endothelium-derived nitric oxide (EDNO/EDRF) (9,10), also known as endothelium-derived relaxing factor (EDRF) (11–13), which activates soluble guanylate cyclase (GC) found in vascular smooth muscle, and endothelium-derived hyperpolarizing factor (EDHF) (14) and prostacycline (PGI₂) (15) which activate the smooth muscle membrane bound enzyme, adenylate cyclase (AC) (11). Other factors such as atrial natriuretic factor (ANF), acetylcholine (Ach) (16), bradykinine (16), endothelin (ET) (6,7), adenosine triphosphate (ATP) (17), and shear-stress (5,18) are also potent dilators which act mainly by as releasers of EDNO and/or EDHF. Ventilation (19) alone induces pulmonary vasodilation.

Vascular smooth muscle cell membrane potential is partly regulated by K⁺ channels (20–22), of which the K_{ATP} channels seem to play a critical role by controlling calcium (Ca²⁺) influx (23–25). Various K⁺ channels are activated by either EDNO (26) or EDHF (14). NO activates soluble GC found in vascular smooth muscle cells, (11) thereby increasing cyclic 3'-5'-guanylosine monophosphate (cGMP). cGMP-dependent protein kinase (PKG) activates calcium-dependent K⁺ channels (K_{Ca}^{2+}) through phosphorylation. The resulting hyperpolarization of cell membrane inhibits the influx through voltage operated Ca²⁺ channels and causes vasodilation (26).

To establish that K_{ATP} channels are present at birth in the fetal pulmonary circulation of lambs with and without CDH

Abbreviations: Ach, acetylcholine; Ao, aorta; CD, congenital diaphragmatic hernia; EDHF, endothelium-derived hyperpolarizing factor; EDNO, endothelium-derived nitric oxide; ET (-1), endothelin (-1); GLI, glibenclamide; K_{ATP} , ATP-dependent potassium; LPA, left pulmonary artery; PAP, pulmonary arterial pressure; PVR, pulmonary vascular resistance

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and that they may play a role in the modulation of pulmonary vascular tone, we studied *in vivo* and *in vitro* the effect of pinacidil, a K_{ATP} channel activator, and of glibenclamide (GLI), a K_{ATP} channel inhibitor. We also try to show by immunohistochemistry the presence of K_{ATP} channels in lungs of lambs with CDH.

MATERIALS AND METHODS

In vivo. The surgical procedure was approved by the Animal Care and Use Committee of the Ecole de Chirurgie, Assistance Publique-Hôpitaux de Paris (France). The operation was performed on pregnant ewes ("Pré-Alpes du Sud") between October, 1998 and February, 2000.

Surgical preparation. The induction of anesthesia of pregnant ewes was realized with pentobarbital sodium. Analgesia was realized by an intrathecal lumbar puncture of 10 mg of Marcaine. The anesthesia was completed with 30 mg per kilogram (kg) of pentobarbital sodium. The ewes were intubated. Heart rate (HR) and oxygen saturation in the blood were continuously recorded (Hewlett Packard, M1165 A., Model 54S, Lorraine, France).

First surgical procedure: Creation of a diaphragmatic hernia. At 85 d of gestation (term 147 d), fetal lambs were delivered through a midline incision of the abdomen and of the uterus (27–29). The left lung was exposed and a 2-cm incision with partial resection was made in the left part of the diaphragmatic muscle. The stomach and the omentum were pushed up into the chest. After fetal chest closure, the lamb was gently replaced in the uterus which was closed.

Second surgical procedure. The second operation was performed after 135 d of gestation on these same ewes and on pregnant ewes which had not undergone the first operation. The first surgical steps were the same as those described above. Polyvinyl catheters were inserted into the axillary artery and the axillary vein and pushed respectively into the aorta (Ao) and the superior vena cava. Three catheters were inserted by puncture, a Tygon 22-Gauge (G) 10 mm into the main pulmonary artery (MPA), a Tygon 20-G 20 mm into the left pulmonary artery (LPA) and a Tygon 20-G 13 mm into the left atrium (LA). Two cuff-type 6-mm ultrasonic flow probes (Transonics, Ithaca, NY) were placed around the Ao and the LPA. The probes were attached to an internally calibrated flowmeter to measure the blood flow in the vessels. Umbilical-placental circulation was preserved. The lambs were allowed a postoperative recovery period of at least one hour before the infusion of drugs. At the end of this procedure the fetal lambs and ewes were killed under anesthesia with a T61 solution (Hoechst Roussel Vet), and the fetuses were weighed. The position of the catheters was controlled at autopsy.

Physiologic measurements. The blood pressure in the MPA, in the LA and in the Ao (respectively PAP, LAP, PAo) was measured with a transducer (Baxter, Bentley Laboratories, Uden, The Netherlands). The atmospheric pressure constituted our pressure of reference. The pulmonary vascular resistance (PVR) was defined as the difference between the PAP and the LAP divided by the LPA blood flow (Mean PAP–Mean LAP/LPA blood flow). The blood flow in the Ao and in LPA (respectively QAo and QAP) was measured with an ultrasonic flow transducer connected to a calibrated flowmeter (Transonic Systems Inc. T206). A conventional ventilator was used (Servo Ventilator 900 B, Siemens Elema, Sweden). Physiologic hemodynamic measurements were recorded every 2 min during 20 s through the whole 20 min procedure. Fetal blood samples were obtained every 20 min from catheter in the superior vena cava to measure the blood gas and glucose level. (Radiometer Copenhagen, Radiometer A/S, Emdrupves 72 DK, 2400 Copenhagen). pH was kept between 7.35 and 7.45 and blood glucose level was considered normal between 3.5 and 7 mM.

Drug preparation. Pinacidil monohydrate (N-cyano-N-4 pyridinyl-N-(1,2,2-trimethylpropyl) guanidine; Leo Pharmaceutical Products Copenhagen, Denmark) was dissolved in DMSO (DMSO) to reach a final concentration of 1 mg/mL. The final pH of this solution was 7.4. GLI ((5-chloro-N-2,4-cyclohexyl-amino-carbonyl-amino-sulfonyl-phenyl-ethyl-2-methoxybenzamide), Sigma Chemical Co. Chemical) was dissolved in DMSO and diluted to a final concentration of 1 mg/mL. The final pH was 8 and the final concentration of DMSO was 1%. DMSO was tested at the same concentration of 1 mg/mL on vascular rings from CDH and non CDH animals in organ baths. DMSO had no effect concerning contraction or dilation of vascular rings.

Experimental records. Different protocols were performed using these different drugs on the same animal. We did not use pinacidil and GLI on the same animal with CDH. The period of recovery allowed between drugs was at least 30 min after ACh and one hour after pinacidil. The sequence of the

drugs was not randomized, GLI was administered last. Drug dosage was determined according to previous studies on fetal lambs (30–31).

Protocol 1. Effect of pinacidil in (a) fetus lambs with CDH and (b) fetus lambs without CDH. Pinacidil (2 mg) was infused for 2 min into the LPA.

Protocol 2. Effect of prolonged infusion of GLI in (a) fetus lambs with CDH and (b) fetus lambs without CDH. GLI (1 mg/min) was infused into the LPA for 30 min.

In vitro: Organ bath experiments. Preparation of vessel rings: Pulmonary lungs were obtained from lambs with and without CDH. Rings of 2 mm length and 200–300- μ m internal diameter (5th–6th generation) were obtained from these vessels. The vascular rings were placed in separate 20-mL organ chambers filled with Krebs and suspended between two 25- μ m tungsten wires. The organ chambers were maintained at 37°C and oxygenated continuously with 95% of O₂ and 5% of CO₂. One of the wires was connected to a force transducer for isometric tension recording (EMKA technologies, France and Maclab computer 6300, Apple, Medford, MA). Isometric tension developed in response to drug stimulation was measured by means of linear tension displacement transducers. Before experimentation, vascular rings were stretched to their predetermined optimal passive load to obtain an optimal length-tension relationship (2 g). Vascular rings were equilibrated for 60 min before experimentation. During this time, the vessels were stretched three times and washed until a functionally relevant tension, as predicated by the Laplace equation, was achieved.

Solutions and drugs. Indomethacin (Sigma Chemical Co. Chemical) was used at a final concentration of 10⁻⁵ M, pH 7.4; Phenylephrine (Sigma Chemical Co. Chemical) at a concentration of 10⁻⁷–10⁻⁵ M, pH 7.4; ACh (2-(Acetyloxy)-N,N,N-Trimethylethanium chloride) at a concentration of 10⁻⁷–10⁻⁴ M, pH 7.4; Sodium nitroprusside at a final concentration of 10⁻⁴ M; Pinacidil monohydrate at a concentration of 10⁻⁵–10⁻⁴ M, pH 7.4; GLI at a concentration of 10⁻⁵ M, pH 8.

Experimental design. Indomethacin 10⁻⁵ was added at the beginning of each protocol. Arteries were precontracted in Krebs solution with phenylephrine 10⁻⁵ M. The effects of contracting agents were measured by the rise in tension over a basal tension and were expressed in absolute values (mM mm⁻¹). The effects of relaxing agents were measured by the fall in tension under a basal tension. The degree of the response was given as the percentage reversal of the tension generated by contracting agents (32).

Protocol 3. Effect of ACh on contracted vessels. Once the effect of phenylephrine was fully developed and contraction response had stabilised (15–20 min), ACh 10⁻⁵ M and then 10⁻⁴ M were added to the bath.

Protocol 4. Effect of pinacidil on contracted vessels. Once the effect of phenylephrine was fully developed and contraction was stabilised, 10⁻⁵ M and 10⁻⁴ M concentrations of pinacidil were added to the bath before washing.

Protocol 5. Effect of GLI on non-contracted vessels. GLI 10⁻⁵ M was added to the bath.

Protocol 6. Protocol 6: Effect of GLI on the response of the arteries to pinacidil. Once the effect of phenylephrine was fully developed and contraction was stabilised, GLI 10⁻⁵ M was added to the bath before washing. Pinacidil was then added to the bath after a suitable interval.

Tissue preparation. Artery rings from lambs with and without CDH were fixed in 10% neutral buffered formalin Standard histologic methods of dehydration were used. Slides were cut in the paraffin wax blocks with a standard microtome (Leica, CM 3000, Germany) at 5 μ m. Other artery rings were fixed on a Tissu-Tek and frozen in a cold chamber at -20°C.

Procedure. The paraffin slides were incubated with rabbit protein-blocking serum during 20 min, with the primary antibody K_{ATP} at 250 μ g/mL for 60 min at 37°, with biotinylated, species-specific secondary antibody (anti-rabbit IgG, Vector Laboratories) at a concentration of 2.25 μ g/mL for 30 min and finally in a peroxidase-conjugated avidin-biotin complex (KIT ABC vectastain, Vector laboratories) for one hour to quench endogenous peroxidase activity. Diaminobenzidine tetrahydrochloride (D.A.B.) (Sigma Chemical Co. Chemical Co) was used as chromogen to reveal antigenic sites.

Statistical analysis: All data are expressed as means \pm SEM in vivo. Each datum represents the mean of the values recorded during a given period: periods of 20 min before the infusion of each drug and periods of four minutes after the infusion of ACh and of 20 min after the infusion of pinacidil or GLI. *In vitro:* Doses-response curves were constructed for each drug. Statistical significance was determined by analysis of variance for repeated measures. The drug concentration which produced one-half of the maximal relaxation response (EC50) was used as an index of tissue sensitivity. The statistical comparisons were established with the help of a Wilcoxon test (Statistica). Differences are considered significant for $p < 0.05$.

RESULTS

In Vivo. Twelve ewes with CDH were analyzed in this study. Of the 19 pregnant ewes, 1 gave birth before the second surgical procedure, 3 proved not to carry a fetus with a diaphragmatic hernia when the second operation was performed, and 1 died during the second surgical procedure. Two ewes were not included in our protocol because of technical problems during instrumentation. Five ewes which carried fetuses without CDH were also analyzed in this study. *N* = number of animals studied. The mean weight for fetal lambs with CDH (*n* = 12) was 2.6 kg ± 0.7, for fetal lambs without CDH (*n* = 5) 3.4 kg ± 0.4. The blood gas values after the injection were comparable to those recorded before the injection. Blood glucose levels controlled every 20 min were normal. Table 1 summarizes the mean values of the responses of the PAP, PAo, PVR, QAP, QAo and HR before and after the injection of Pinacidil or GLI in fetal lambs without CDH and in fetal lambs with CDH.

Protocol 1. (Table 1) Animals received 2 mg of pinacidil which corresponds to 0.6 10⁻⁵ M/kg for lambs without CDH (*n* = 5) and to 0.3 10⁻⁵ M/kg for lambs with CDH (*n* = 6). There was a significant reduction of PAP and PVR after the injection of pinacidil in fetus lambs with and without CDH.

Protocol 2. (Table 1) Animals received 30 mg of GLI over a period of 30 min, which corresponds to 0.9 10⁻⁵ M/kg for lambs without CDH (*n* = 5) and to 0.8 10⁻⁶ M/kg/min for lambs with CDH (*n* = 6). There was an insignificant increase of PAP and PVR after the injection of GLI in fetus lambs with CDH.

In Vitro. Organ Bath Experiments. Protocol 3. (Fig. 1). The relaxing effect of ACh on arteries taken from lambs with CDH (*n* = 5) was insignificant. The response varied with the concentration used, with a 0.1-μM dose having no effect and a 10-μM dose producing an insignificant relaxation. ACh had a significant relaxing effect on arteries taken from lambs without CDH (*n* = 5). The degree of relaxation was dependent on the concentration used, with the 0.1-μM dose having no effect and the 10-μM dose inducing marked relaxation.

Protocol 4. (Fig. 1). The relaxing effect of pinacidil on arteries taken from lambs with and without CDH (in both

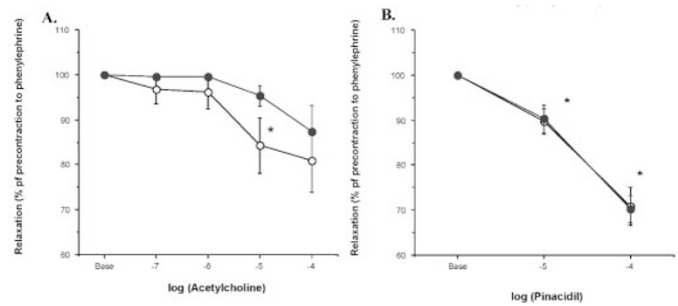


Figure 1. Compared relaxing responses in organ baths in response to phenylephrine (1 μM) by isolated pulmonary artery rings taken from lambs without (*n* = 5) and with CDH (*n* = 6) after the injection of Ach (0.1 μM–0.1 mM) (A) and after injection of pinacidil (10 μM–0.1 mM) (B). **p* < 0.05.

samples *n* = 5) was significant, with 0.1-μM dose producing a significant relaxation. Fig. 2 shows the greater relaxing response of arteries taken from lambs with and without CDH after the injection of pinacidil compared with the Ach-induced relaxing response.

Protocol 5. GLI itself had no effect on ring segments of arteries in organ chamber.

Protocol 6. GLI at a concentration of 10⁻⁵ M did not inhibit the relaxation of pinacidil (10⁻⁵ M) on arteries taken from lambs with and without CDH (in both samples *n* = 5).

Immunohistochemistry. Immunoperoxidase staining for K_{ATP} in fetal lungs of near full-term lambs with and without CDH (Fig. 3) confirmed the presence of K_{ATP} channels in the pulmonary vessels. Immunostaining of the pulmonary parenchyma was only possible using frozen section of the pulmonary parenchyma. Panel C (Fig. 3) shows an absence of nonspecific staining with the secondary antibody in the absence of the primary antibody (control).

DISCUSSION

In this study, activation of K_{ATP} channels by pinacidil induced pulmonary vasodilation with an increased blood flow and a decreased PVR in near full-term lambs with and without CDH. Inhibition of K_{ATP} channels by GLI significantly increased PVR and PAP in near full-term lambs without CDH

Table 1. Mean hemodynamic values recorded in fetal lambs without (*n*=5) and with CDH (*n*=6) before (Baseline) and after (Drugs) the start of drug infusion

	PAP mmHg	PAo mmHg	PVR mmHg/mL/min	QAP mL/min	QAo mL/min	HR Bpm
Pinacidil in fetal lambs without CDH						
Baseline	48.7 ± 5.3	57.4 ± 6.7	0.26 ± 0.04	177.9 ± 24.3	255.4 ± 11.5	133.1 ± 11.6
Drugs	37.0 ± 3.2	52.5 ± 4.4	0.12 ± 0.04	256.9 ± 23.9	312.1 ± 22.2	136.3 ± 16.0
Pinacidil in fetal lambs with CDH						
Baseline	43.7 ± 3.4	44.4 ± 2.9	0.30 ± 0.08	149.6 ± 13.5	126.6 ± 8.4	191.6 ± 9.4
Drugs	36.3 ± 2.9	35.4 ± 2.0	0.14 ± 0.04	201.5 ± 12.7	156.6 ± 11.5	198.3 ± 13
GLI in fetal lambs without CDH						
Baseline	44.6 ± 4.7	52.3 ± 5.2	0.22 ± 0.04	171.5 ± 16.7	318.2 ± 14.2	140.2 ± 15.6
Drugs	53.1 ± 4.2	61.1 ± 4.3	0.27 ± 0.04	194.8 ± 21.9	331.6 ± 19.4	154.5 ± 9.9
GLI in fetal lambs with CDH						
Baseline	50.7 ± 3.8	49.2 ± 5.7	0.38 ± 0.08	154.2 ± 6.7	161.2 ± 9.02	160.2 ± 6.9
Drugs	54.7 ± 5.5	51.6 ± 6.9	0.60 ± 0.12	134.4 ± 7.7	143.9 ± 10.1	151.5 ± 9.2

Values are means ± SEM.

CDH, congenital diaphragmatic hernia; PAP, pulmonary arterial pressure; PAo, aortic pressure; PVR, pulmonary vascular resistance; QAP, pulmonary blood flow; QAo, aortic blood flow; HR, heart rate. ACh, acetylcholine; pinacidil; GLI, glibenclamide.

* Differences are considered significant for *P* < 0.05.

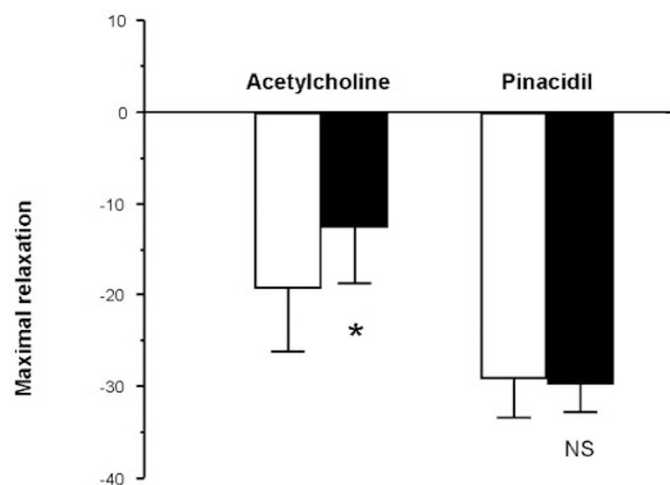


Figure 2. Relaxing responses in organ baths in response to phenylephrine ($1 \mu\text{M}$) by isolated pulmonary artery rings taken from lambs without ($n = 5$) and with CDH ($n = 5$) after the injection of GLI (10^{-5} M) and of ACh or pinacidil. $*p < 0.05$

only. *In vitro*, an ACh-induced relaxation response was present in vascular rings from lambs without (significant) and with CDH (insignificant). The K_{ATP} -opener pinacidil caused concentration dependent relaxation of vascular rings from lambs with and without CDH (both significant). GLI, a K_{ATP} -blocker, did not block the vasodilation induced by pinacidil at the same concentration of 10^{-5} M .

Several types of K^+ channels are implicated in the pulmonary vascular dilation at birth (33–35). The activation of K^+ channels causes K^+ efflux, hyperpolarization of vascular smooth muscle cell membrane, and inhibition of Ca^{2+} influx resulting in relaxation of vascular smooth muscle (33). The main K^+ channels are: 1) Voltage-sensitive K^+ channels (A-channel, delayed rectifier, rapid delayed rectifier, slow delayed rectifier or sarcoplasmic reticulum channels, respectively K_{A} , K_{V} , K_{dr} , K_{ds} , K_{SR}) (34); 2) Ca^{++} -dependent K^+ channels (high, intermediate, small conductance, respectively BK_{Ca} , IK_{Ca} , SK_{Ca}); and 3) ATP-dependent K^+ channels (K_{ATP}). In this study, we specifically investigated K_{ATP} channels. K_{ATP} channel is a hetero-octameric protein of pore-forming subunits, composed of inwardly rectifying K^+ channel (K_{ir}) and sulfonylurea receptor (SUR) subunits (36,37). These channels are found in different tissues, including pancreatic β cells, cardiac, brain, skeletal and smooth muscle cells and are activated by a number of synthetic vasodilators such as pinacidil and are inhibited by oral hypoglycemic sulfonylurea drugs such as GLI. The mechanism of the K_{ATP} channel opening is the result of the fixation of an opener on the channel regulatory subunit SUR, which is an ATP-binding cassette protein (37). The pulmonary vasodilator effect of pinacidil was attributed to the direct activation of K_{ATP} channels on smooth muscle cells with a decrease of intracellular Ca^{2+} concentration or to the activation of endothelial K_{ATP} channels with activation of EDNO (38). Chang (30) showed that K_{ATP} channels play an important role in the modulation of pulmonary vascular tone in newborn lambs through EDNO production. In this study, pinacidil significantly decreased PAP, PAO, and PVR while significantly increasing QAP and

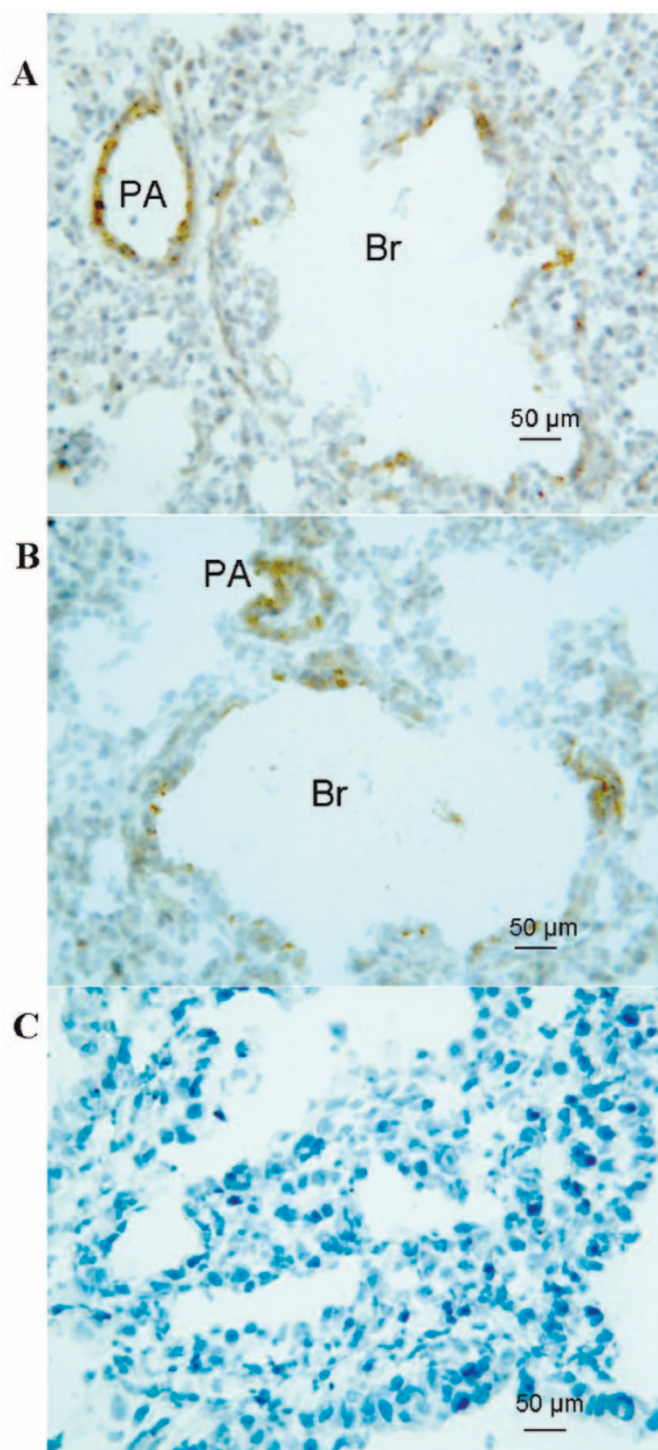


Figure 3. Immunohistochemistry of K_{ATP} in fetal pulmonary arterial wall and fetal bronchioles of near full-term lambs without (A) and with CDH (B). Panel C shows an absence of nonspecific staining with the secondary antibody in the absence of the primary antibody (control). PA, pulmonary arteriole; Br, bronchiole.

QAo in animals with and without CDH. Therefore, we conclude that the K_{ATP} channels are present and respond to stimulation by relaxing pulmonary vessels. If some authors showed that the efficacy of pinacidil is independent of EDNO (38), Luckhoff (23) showed that pinacidil provokes a hyperpolarization of the endothelial membrane through the activa-

tion of K_{ATP} channels resulting in an increase of Ca²⁺ influx and the stimulation of EDNO. GLI did not significantly modify PAP or PVR in near full-term lambs with CDH. We therefore cannot conclude that the inhibition of the K_{ATP} channels has a significant effect in lambs with CDH, although it did modify QAO and QAP. In fetal lambs born without CDH, results are controversial. Chang (30) showed that GLI produces vasoconstriction and an increase in pulmonary and systemic arterial pressure without altering the QAP. Cornfield (31) showed that GLI, although it increases the systemic pressure, has no effect on the basal fetal pulmonary vascular tone. In our study, we obtained a significant increase of PAP, PAO and PVR and a significant decrease of QAP and QAO. The vasodilating capacity of ACh is known to be endothelium-dependent (13). It stimulates the muscarinic M₁ and M₃ receptor-subtypes located on the endothelial cell membrane (16). In our study, the effect of ACh on fetal lambs with CDH was a significant decrease in PAP and PAO, a slight, but not significant, decrease in PVR and an increase in QAP and QAO (Fig. 1).

We are well aware of the several limitations which affected this study. We had to administer several different drugs to the same animal as we did not have enough ewes at our disposal. However, we did not use pinacidil and GLI on the same animal with CDH. The inhibition of the vasodilation effect of pinacidil by GLI is probably dependent on the concentration of GLI. GLI was diluted in DMSO at 10⁻⁵ M and it was not possible reduce dilution to achieve a concentration of GLI higher than 10⁻⁵ M (using DMSO). The concentration of pinacidil was 10⁻⁵. It is therefore possible that the lack of inhibitory effect of GLI on pinacidil is due to insufficient dose of inhibitor to compete with pinacidil rather than an unlikely extra KATP channel effect of pinacidil in our setting

In vitro, ACh caused a significant relaxation in vascular rings taken from lambs without CDH. This relaxation was present but not significant in vascular rings taken from lambs with CDH. This difference can be explained by the fact that the diameter of the lumen of the vascular rings taken from lambs with CDH is particularly narrow; the endothelium is very thin and fragile and is easily rubbed off when the rings are suspended on the tungsten wires. Pinacidil produced a significant relaxation of the vascular rings taken from lambs with and without CDH. This relaxation was always present in vascular rings, thus supporting the hypothesis that pinacidil, a K_{ATP} channel activator, has at least a direct action on the muscular layer of the vascular rings. GLI at a concentration of 10⁻⁵ M did not itself induce any vasoconstriction and did not modify the response to relaxation by pinacidil 10⁻⁴ M and 10⁻⁵ M in rings from lambs with and without CDH.

In conclusion, K_{ATP} channels are present in the pulmonary vessel wall of near full-term lambs with and without CDH (Fig. 3). Pinacidil induced a pulmonary vasodilation and was able to decrease PVR. This vasorelaxation was significant *in vivo* and was observed also in organ bath experiments with vascular rings. Pinacidil appears to have a direct action on the activation of K_{ATP} channels in the vascular smooth muscle cells, although the activation of endothelial K_{ATP} channels may also result from the production of EDNO. EDNO and/or

EDHF appear to be functional at birth in fetal lambs with CDH because the injection of ACh modified the pulmonary vascular tone *in vivo*. GLI increased PVR *in vivo* by decreasing QAP. However, GLI at a concentration of 10⁻⁵ M was unable to modify vascular tone in organ bath experiments and furthermore was unable to block the vasodilating action of pinacidil at a concentration of 10⁻⁵ M and 10⁻⁴ M. Our observations provide the basis for potential therapeutic use of K_{ATP} channels openers to treat pulmonary hypertension caused by CDH.

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