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α_{2B}-Adrenoceptor Deficiency Leads to Postnatal Respiratory Failure in Mice*[S]

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α_2-Adrenoceptors belong to the family of adrenergic receptors, which regulate the neuronal release of norepinephrine as part of a negative feedback loop. Among the α_2-adrenoceptors, the α_{2B}-subtype may also influence developmental signaling pathways involved in angiogenesis of the placenta. Thus, the aim of the present study was to determine whether α_{2B}-adrenoceptors are also involved in other developmental processes beyond placental angiogenesis. Ablation of α_{2B}-adrenoceptors led to lethality of mutant mice during the first hours after birth. Despite normal breathing and drinking behavior, mutant mice developed cyanosis, which could be traced back to a defect in lung morphology with significantly reduced alveolar volume and thickened interalveolar septi. In α_{2B}-deficient lungs and in isolated alveolar type II cells, expression of sonic hedgehog (SHH) was significantly increased, resulting in mesenchymal proliferation. *In vitro* α_{2B}-adrenoreceptor stimulation suppressed expression of sonic hedgehog and the cell cycle genes cyclin D1 and Ki67. *In vivo* inhibition of enhanced SHH signaling by the smoothened antagonist cyclopamine partially rescued perinatal lethality, lung morphology, and altered gene expression in mutant mice. Thus, α_{2B}-adrenoceptors in lung epithelia play an important role in suppressing sonic hedgehog-mediated proliferation of mesenchymal cells and thus prevent respiratory failure.

α_2-Adrenoceptors belong to the family of G protein-coupled receptors that mediate the biological actions of the endogenous catecholamines noradrenaline and adrenaline (1, 2). These receptors were initially identified as presynaptic inhibitory feedback regulators of noradrenaline release from sympathetic or central adrenergic nerve terminals (3, 4). However, α_2-adrenoceptors have since also been found to mediate a wide spectrum of postsynaptic functions including hypotension and bradycardia, sedation, and analgesia (5–8). In addition to the modulation of physiological functions in the adult organism, the α_{2B}-receptor subtype also plays an important role during embryonic development (9, 10).

Mice lacking functional α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors on a mixed genetic background died between embryonic days 9.5 and 11.5 from a severe defect in yolk sac and placental development (10). This defect was mostly ascribed to loss of α_{2B}-adrenoreceptor function as α_{2B}-deficient mice, which were backcrossed onto a C57BL/6 background, showed a similar defect in placenta vasculogenesis (9).

The purpose of the present study was to identify the function of α_{2B}-adrenoceptors in embryonic and perinatal development in mice. Here we demonstrate that α_{2B}-deficient mice suffer from an early postnatal defect in lung maturation. In α_{2B}-deficient lungs, sonic hedgehog (SHH)[2] and its receptor patched were up-regulated, resulting in enhanced mesenchymal proliferation. *In vivo* inhibition of SHH signaling by the smoothened antagonist cyclopamine partially rescued postnatal lethality and the pulmonary phenotype associated with α_{2B} ablation. These findings indicate that α_{2B}-adrenoceptors play an essential role for pulmonary development by suppressing sonic hedgehog signaling.

EXPERIMENTAL PROCEDURES

Generation of α_{2B}-Adrenoceptor-deficient Mice—The targeted deletion of the α_{2B}-adrenoceptor gene (*Adra2b*$^{-}$) has been described previously (7). For this study, *Adra2b*$^{-/-}$ mice, which were backcrossed onto a C57BL/6 background (Harlan Winkelmann, Borchen, Germany) for 12 generations, were used (9). All animal procedures were approved by the responsible animal care committee of the University of Freiburg, Germany. Genotyping was performed with primers as described previously (9). Cyclopamine (18 mg/kg of body weight, Sigma-Aldrich, Taufkirchen, Germany) was applied subcutaneously to pregnant mice.

Histology and Immunochemistry—Lungs and hearts were fixed in 4% paraformaldehyde and embedded in paraffin or araldite. Cardiac sections were stained with hematoxylin and eosin. Semithin araldite sections of the lung were stained with methylene blue (11, 12). For immunodetection of sonic hedgehog, cryostat sections were incubated with primary antiserum (R&D Systems, Wiesbaden, Germany) followed by an Alexa Fluor 488 secondary antibody (Molecular Probes, Invitrogen, Darmstadt, Germany). Nuclei were visualized by brief incubation with 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Blood slides were stained with Giemsa and May-Grünwald solutions (Carl Roth, Karlsruhe, Germany).

Isolation and Cultivation of Primary Cells—Alveolar type II cells were isolated as described with modifications (13). Cell

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables 1 and 2.

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[2] The abbreviations used are: SHH, sonic hedgehog; P, postnatal day; E, embryonic day; ATII, alveolar type II.

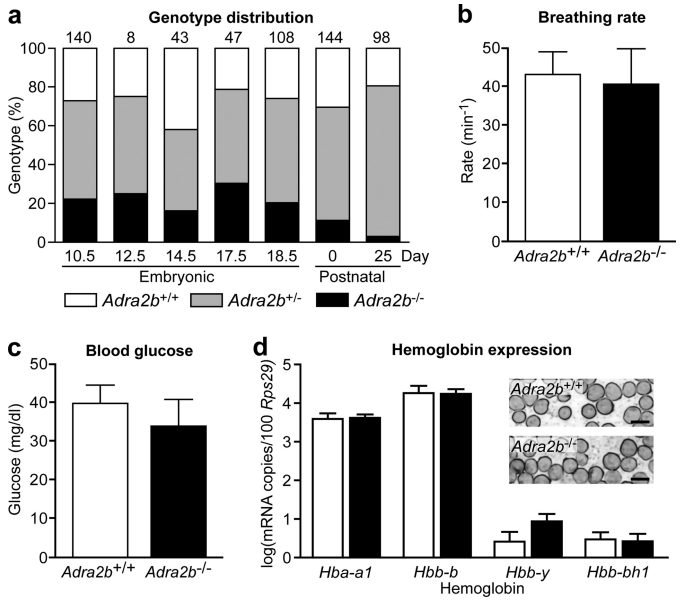

FIGURE 1. **Perinatal survival and characterization of α_{2B}-adrenoceptor-deficient mice.** *a*, genotype distribution of the targeted α_{2B}-allele (*Adra2b*$^{-}$) was determined during the embryonic and perinatal period in mice derived from heterozygous intercrosses. *Numbers* above columns indicate the total number of mice investigated at the respective time points. *b* and *c*, spontaneous breathing rate (*b*) and blood glucose (*c*) did not differ between wild-type and mutant mice immediately after birth (n = 8–14 per genotype for breathing rate, n = 18–21 for blood glucose). *d*, erythrocyte morphology (*inset*: Pappenheim staining; *bars*, 10 μm) and mRNA expression of hemoglobin chains in the liver on postnatal day P0 did not differ between genotypes (n = 5–6 per genotype).

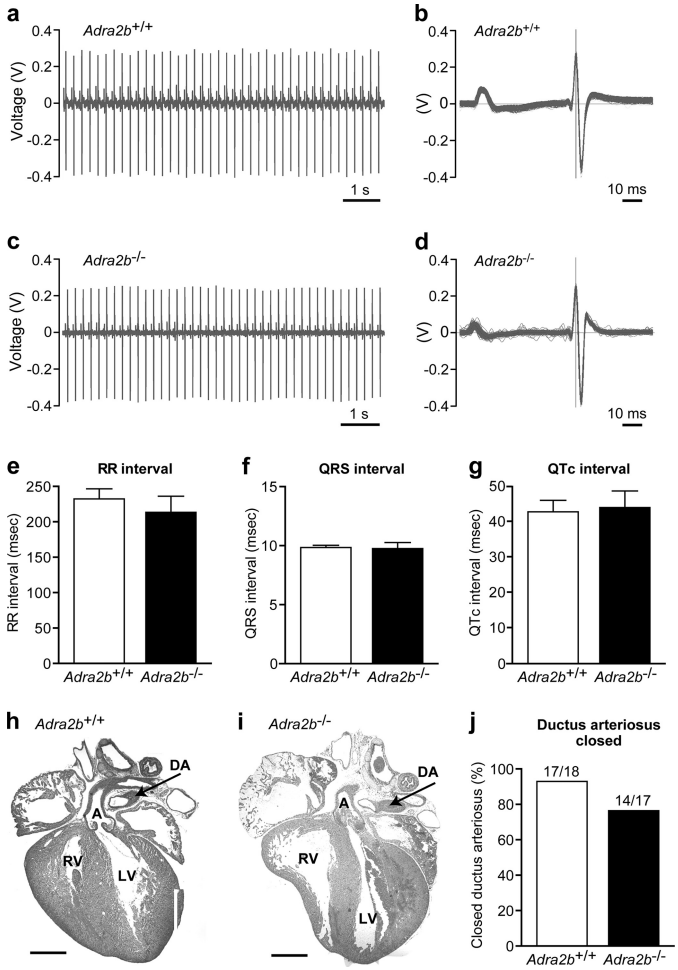

FIGURE 2. **Cardiac function and structure in α_{2B}-adrenoceptor-deficient mice on postnatal day 0.** *a–g*, electrocardiograms were recorded from α_{2B}-deficient and wild-type mice during isoflurane anesthesia on postnatal day 0. *Adra2b*$^{-/-}$ mice showed regular rhythm (*c* and *d*) and normal averaged ECGs (*b* and *d*), RR interval (*e*), QRS interval (*f*), and QTc interval (*g*, n = 8–13 per genotype). *h* and *i*, frontal sections through hearts on day P0 revealed normal cardiac structure (hematoxylin and eosin staining; *bars*, 500 μm) and closure of the ductus arteriosus (*A*, aorta; *DA*, ductus arteriosus (*arrow*); *LV*, left ventricle; *RV*, right ventricle). *j*, ductus arteriosus closure in *Adra2b*$^{+/+}$ and *Adra2b*$^{-/-}$ mice on day P0 (n = 17–18 per genotype, p = 0.34, Fisher's test).

suspensions from bronchoalveolar lavage of neonatal mice were passed through 100-, 40- (BD Biosciences, Heidelberg, Germany), and 20-μm (Millipore, Schwalbach, Germany) filters and incubated with biotinylated anti-CD31, anti-CD45, and anti-CD16/32 antibodies (BD Biosciences). Then streptavidin-coated magnetic particles (Biotin Binder Dynabeads, Invitrogen) were added. Magnetic particles were captured using a magnetic tube separator (Promega, Mannheim, Germany), and the remaining cells were plated on tissue culture dishes for 16 h to remove further contaminating cells. Cell suspensions were finally seeded on fibronectin-coated glass coverslips. After 4–6 h of cultivation, cells were used for gene expression analysis or were cultivated for an additional 16 h and stained with methylene blue for cell identification.

Gene Expression—Total RNA was prepared from lung samples and primary alveolar type II cells with the RNeasy kit (Qiagen, Hilden, Germany). For quantitative real-time PCR analyses, 35 μl of the amplification mixture (Qiagen, QuantiTect SYBR Green kit) was used containing 20 ng of reverse-transcribed RNA and 300 nmol/liter primers (supplemental Table 1, Eurofins MWG Operon, Ebersberg, Germany). Reactions were incubated in triplicate on an MX3000P detector (Agilent Technologies, Waldbronn, Germany). The cycling conditions were: 15 s of polymerase activation at 95 °C and 40 cycles at 95 °C for 15 s, at 58 °C for 30 s, and at 72 °C for 30 s (5, 14).

Cardiac Function and Blood Glucose—Electrocardiograms were recorded from newborn mice during isoflurane anesthesia (DSI Transoma Medical, TA10EA-F20 transmitters) as described previously (11, 15). Recordings were obtained at a sampling frequency of 2 kHz using the ECG module of ADI Chart software (AD Instruments, Castle Hill, Australia). Blood glucose was determined using an AccuChek sensor (Roche Applied Science, Grenzach-Wyhlen, Germany).

Statistical Analysis—Data are presented as means ± S.E. of individual data points. Data were analyzed using one- or two-way analysis of variance followed by Bonferroni post hoc tests or Student's t test, respectively, if not indicated otherwise. A p value of less than 0.05 was considered as statistically significant.

RESULTS

Perinatal Lethality of α_{2B}-Adrenoceptor-deficient Mice—Heterozygous intercrosses of α_{2B}-adrenoceptor-deficient mice resulted in a percentage of *Adra2b*$^{-/-}$ mice at weaning age that was significantly lower than the predicted Mendelian ratio (Fig. 1*a*, 3 *Adra2b*$^{-/-}$ out of 98 offspring at postnatal day P25). Between embryonic days E10.5 and E18.5, however, the genotype distribution did not deviate significantly from the pre-

dicted Mendelian ratio. Most α_{2B}-deficient mice died during the day of birth (Fig. 1*a*). Newborn α_{2B}-deficient mice showed normal breathing rate (Fig. 1*b*) and drinking behavior, as evidenced by the presence of visible milk in their stomach. At this time, blood glucose levels did not differ between genotypes (Fig. 1*c*). No alterations in hematopoiesis could be identified as erythrocyte counts and morphology were normal in α_{2B}-deficient mice on postnatal day 0 (Fig. 1*d*). Hepatic mRNA expression of hemoglobin chains did not differ early after birth (Fig. 1*d*). However, during the next hours, *Adra2b*$^{-/-}$ mice were retarded in their weight gain and became cyanotic.

TABLE 1
Body and organ weights of newborn *Adra2b*$^{-/-}$ and *Adra2b*$^{+/+}$ mice (P0)

	Adra2b$^{+/+}$	*n*	*Adra2b*$^{-/-}$	*n*	*p*
Body weight P0 (g)	1.322 ± 0.026	31	1.004 ± 0.023	24	0.0001
Heart weight P0 (g)	0.008 ± 0.001	11	0.007 ± 0.001	6	0.1796
Lung weight P0 (g)	0.029 ± 0.001	9	0.019 ± 0.002	4	0.0001
Liver weight P0 (g)	0.044 ± 0.003	11	0.038 ± 0.003	6	0.1786
Kidney weight P0 (g)	0.013 ± 0.001	4	0.012 ± 0.001	6	0.0922

Cardiac Phenotyping of α_{2B}-deficient Mice—Cardiac structure and function did not differ between wild-type and α_{2B}-mutant mice on postnatal day 0 (Fig. 2). ECG recordings revealed normal cardiac rhythm with no signs for conduction deficits in α_{2B}-deficient mice after birth (Fig. 2, *a–g*). Upon microscopical investigation of *Adra2b*$^{-/-}$ hearts, all valves and chambers were properly developed, and postnatal closure of the ductus arteriosus Botalli was normal (Fig. 2, *h–j*, $p = 0.34$). However, the right ventricle was significantly dilated in *Adra2b*$^{-/-}$ as compared with wild-type hearts (internal diameter in *Adra2b*$^{+/+}$, 564.8 ± 58.3 μm, $n = 5$, *versus Adra2b*$^{-/-}$, 937.4 ± 87.4 μm, $n = 4$, $p < 0.01$). Organ weights of heart, kidney, and liver were similar between genotypes, but α_{2B}-deficient mice had significantly lower body and lung weights than wild-type mice (Table 1).

Pulmonary Phenotype of α_{2B}-deficient Mice—Reduced lung weight, cyanosis, and dilatation of the right ventricle led us to investigate pulmonary development and morphology (Fig. 3). Between embryonic days E14.5 and E18.5, lungs from *Adra2b*$^{-/-}$ mice developed normally, and cellular *versus* alveolar/bronchial spaces were similarly distributed between genotypes (Fig. 3, *a–l*). However, early after birth, lungs of *Adra2b*$^{-/-}$ mice were less inflated and showed reduced alveolar spaces and thickened interalveolar septi (Fig. 3, *j–l*). Capillary density did not differ between genotypes. *Adra2b*$^{-/-}$ lungs were characterized by a significantly higher degree of mitosis and increased expression of the cell cycle markers cyclin D1 (*Ccnd1*) and Ki67 (*Mki67*) (Fig. 4, *a–c*). Quantitative real-time PCR revealed that α_{2B}-adrenoceptors were expressed at 3.3- and 3.7-fold higher levels in the lung as compared with α_{2A}- or α_{2C}-adrenoceptors, respectively (Fig. 4*d*). To determine the cellular localization of α_{2B}-adrenoceptors, bronchial epithelia, which stained positively for expression of SHH and interstitial lung tissue, were microdissected and subjected to quantitative real-time PCR analysis (Fig. 4*e*). Bronchial specimens showed 5-fold higher α_{2B}-mRNA expression than interstitial cells, suggesting that

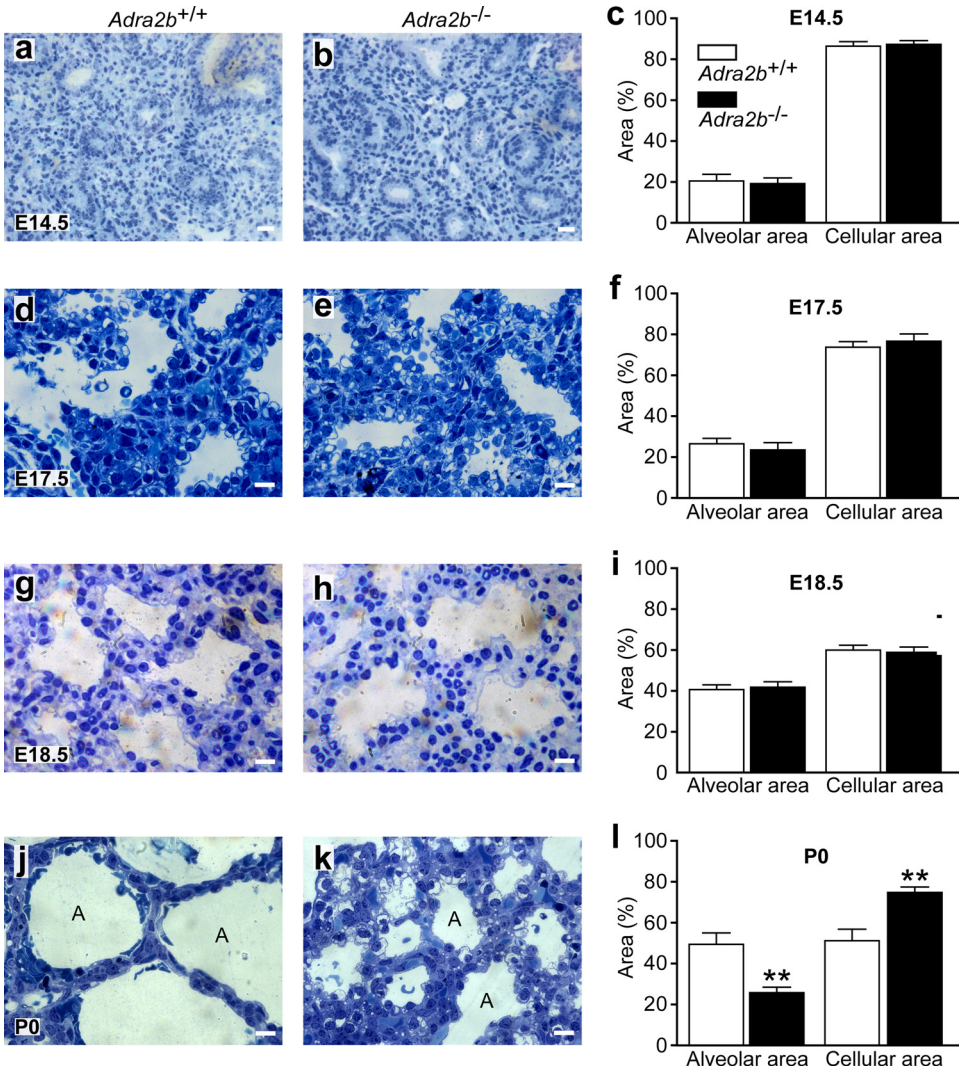

FIGURE 3. **Histology and morphometric analysis of lungs in α_{2B}-adrenoceptor-deficient mice between embryonic day E14.5 and postnatal day P0.** *a–l*, lungs from *Adra2b*$^{-/-}$ and wild-type mice were obtained from timed matings at the indicated days of development. Morphometric analysis of methylene blue-stained lung sections revealed normal distribution of alveolar/bronchial spaces *versus* cellular areas during embryonic development (*c*, *f*, and *i*) but increased interalveolar septi and cell areas in *Adra2b*$^{-/-}$ lungs on postnatal day P0 (*A*, alveoli; *bars*, 20 μm, E14.5 and 10 μm, E17.5–P0; $n = 3–7$ per genotype and time point, **, $p < 0.01$ *versus Adra2b*$^{+/+}$).

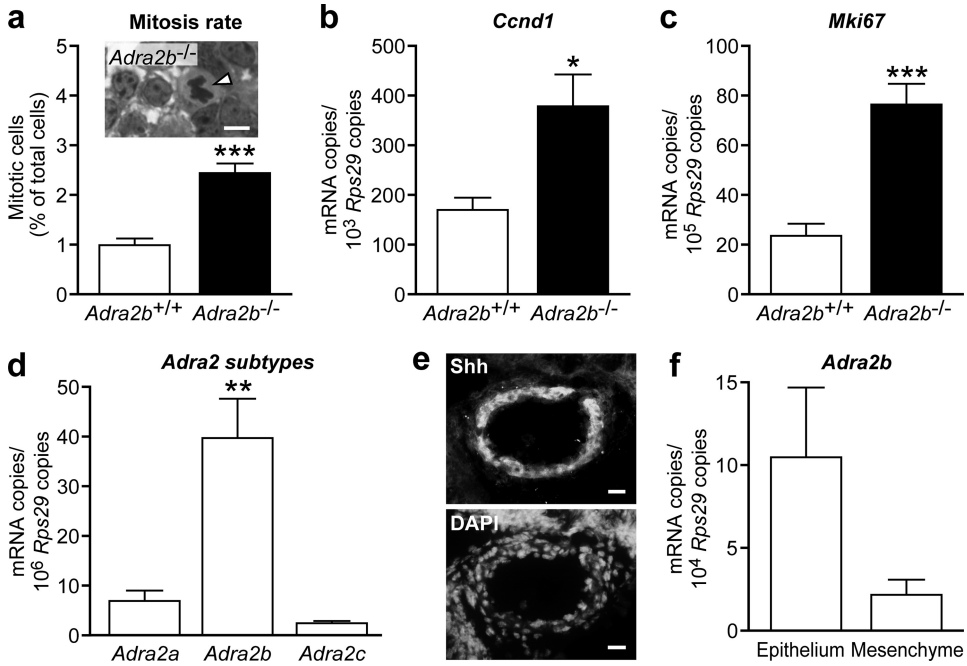

FIGURE 4. **Rate of mitosis, cell cycle gene expression, and α_2-adrenoceptor subtype distribution in the lung.** *a*, *Adra2b*$^{-/-}$ lungs contained significantly more mitotic cells (*inset*, *arrowhead*) as compared with wild-type lungs on postnatal day 0 (*bar*, 10 μm). *b* and *c*, mRNA expression of cell cycle regulators cyclin D1 (*Ccnd1*, *b*) and Ki67 (*Mki67*, *b*) was significantly higher in *Adra2b*$^{-/-}$ lungs on day P0 (*a–c*, $n = 5$ per genotype, *, $p < 0.05$, ***, $p < 0.001$ *versus Adra2b*$^{+/+}$). *d*, α_{2B}-adrenoceptors were expressed at higher mRNA levels in the wild-type lung on day P0 than α_{2A}- or α_{2C}-adrenoceptors (*d*, $n = 4–6$ per genotype, **, $p < 0.01$ *versus Adra2a*). *e* and *f*, immunostaining of SHH in bronchial epithelia of the wild-type lung on day P0. *e*, *lower panel*, DAPI fluorescence to detect nuclei (*bars*, 20 μm). *f*, α_{2B}-adrenoreceptor mRNA expression in bronchial epithelial cells and interstitial cells after microdissection from cryostat sections of wild-type lungs on day P0 ($n = 4$ per group).

α_{2B}-receptors are coexpressed with SHH in epithelial cells of the lung (Fig. 4*f*).

Gene Expression in α_{2B}-deficient Lungs on Postnatal Day 0—To search for the mechanism of the defect in perinatal lung development in mutant mice, genes that have previously been shown to play an essential role for lung development were determined in their expression on day P0 (supplemental Table 2). Out of 40 genes tested, 6 were increased in their expression in *Adra2b*$^{-/-}$ lungs, including *Ccnd1*, *Flt1*, *Mki67*, *Ptch1*, *Shh*, and *Vegfa* (supplemental Table 2). Of these genes, sonic hedgehog (*Shh*) was most strongly up-regulated in *Adra2b*$^{-/-}$ *versus* wild-type lung tissue (supplemental Table 2). Also, the SHH receptor patched (*Ptch1*) was significantly increased in its expression (supplemental Table 2). However, smoothened (*Smo*), which interacts with patched, did not differ in its expression between genotypes (supplemental Table 2).

Gene Expression in α_{2B}-deficient Alveolar Type II (ATII) Cells on Postnatal Day 0—To test whether *Shh* is regulated in epithelial cells, ATII cells were isolated on postnatal day 0. To validate the cell isolation procedure, the expression of *Ddr2*, a fibroblast marker (16), *Pecam*, an endothelial marker (17), and *Spb*, an ATII marker (18), was assessed. Relative to *Spb*, expression of *Pecam* and *Ddr2* was significantly reduced in primary cultured ATII cells as compared with intact lungs, confirming enrichment of ATII cells (Fig. 5*a*). Light microscopic analysis confirmed the purity of the cultivated ATII cells (18, 19) and did not indicate morphological differences between genotypes (Fig. 5*b*). The expression of *Shh* mRNA was 3-fold higher in *Adra2b*$^{-/-}$ *versus* wild-type ATII cells (Fig. 5*c*).

α_{2B}-Adrenoceptor Stimulation Suppresses Shh Expression—We hypothesized that α_{2B}-receptors might modulate expression of *Shh* to control perinatal lung development. Lung slices from wild-type and α_{2B}-deficient E17.5 embryos were maintained in DMEM *in vitro* and were stimulated with the α_2-agonist medetomidine (Fig. 5, *d–i*). Medetomidine suppressed *Shh* mRNA expression in wild-type but not in α_{2B}-deficient lungs (Fig. 5*d*). Furthermore, SHH protein levels were lower in lung specimens that were cultivated in the presence of medetomidine (Fig. 5*e*). Lung section histology was not affected by the *in vitro* culture (Fig. 5*f*). Expression of cyclin D1 (*Ccnd1*) and the sonic hedgehog target genes *Gli1* and *Gli2* but not *Gli3* was significantly reduced by the α_2-adrenoceptor agonist medetomidine (Fig. 5, *g* and *i*).

Cyclopamine Rescue of α_{2B}-mediated Pulmonary Phenotype—To test whether dysregulated *Shh* expression might be causally related to the pulmonary phenotype in α_{2B}-deficient mice, pregnant mice from heterozygous crosses were treated with the smoothened antagonist cyclopamine (20, 21) starting on embryonic day E17.5. Cyclopamine treatment significantly increased the percentage of mutant mice surviving the immediate perinatal period (Fig. 6*a*, $p = 0.017$). One day after birth (P1), lung histology of cyclopamine-treated α_{2B}-deficient mice did not differ from wild-type lungs (Fig. 6, *b–d*). Furthermore, expression of *Shh* and the cell cycle markers *Ccnd1* and *Mki67* was significantly reduced in *Adra2b*$^{-/-}$lungs after cyclopamine treatment (Fig. 6, *e–g*).

DISCUSSION

The main finding of the present study is that α_{2B}-adrenoceptors are essential for perinatal survival and lung development in mice. α_{2B}-Adrenoceptors may modulate the interaction between epithelial and mesenchymal cells by suppressing sonic hedgehog expression and signaling (Fig. 7). Ablation of α_{2B}-adrenoceptor expression led to early postnatal respiratory failure.

α_{2B}-Adrenoceptors contribute to presynaptic inhibition of neurotransmitter release (22) and elicit hypertension and vasoconstriction as postsynaptic receptors (7, 23, 24). In addition, the α_{2B}-subtype is unique among the adrenoceptors because it plays an essential role during embryonic development (9, 10). Although previous studies have demonstrated the involvement of α_{2B}-receptors in extraembryonic development (9, 10), *i.e.* placenta and yolk sac, the present study shows that α_{2B}-recep-

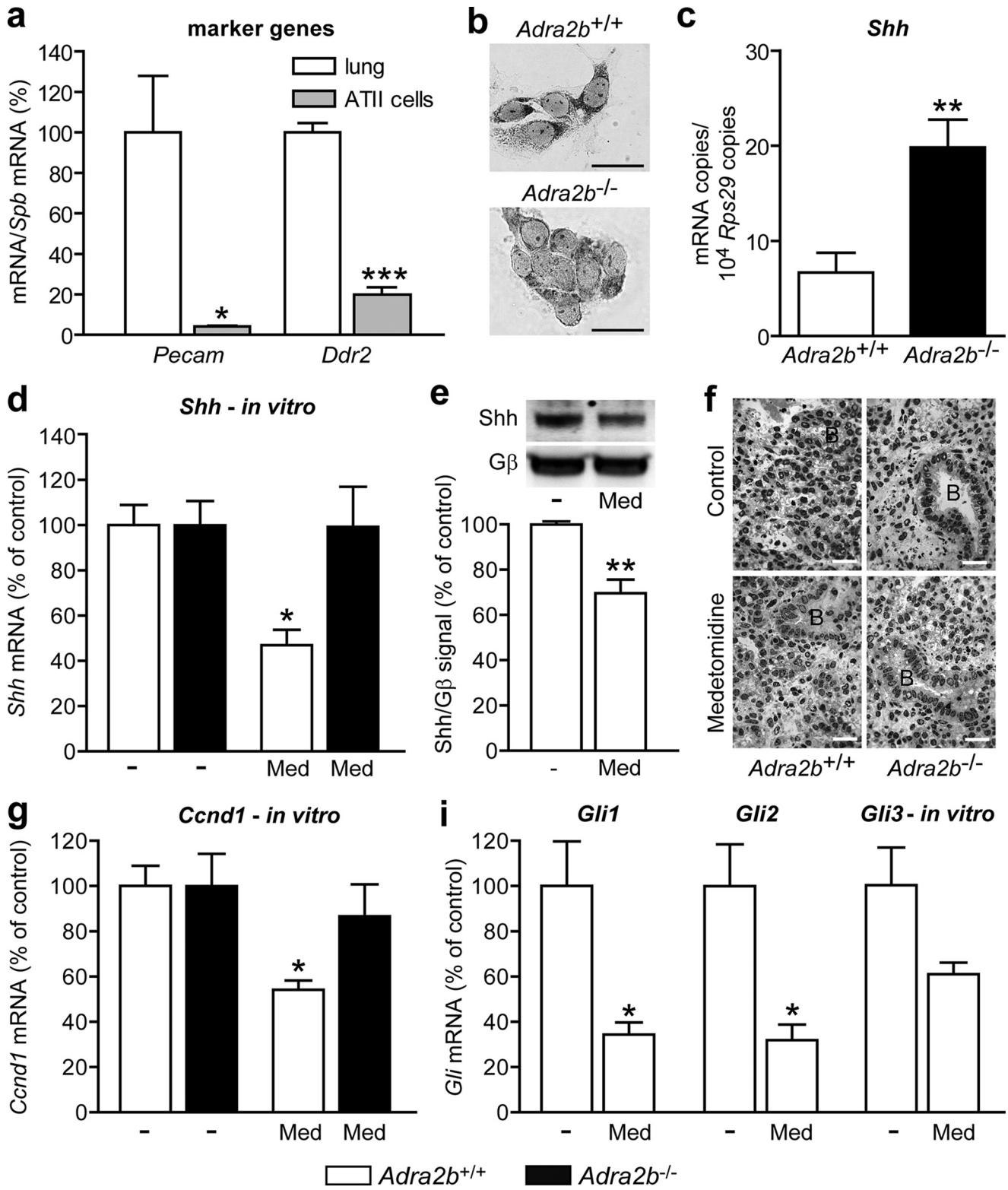

FIGURE 5. **Expression of *Shh* and downstream signaling molecules in primary alveolar type II cells and in the lung *in vitro*.** *a–c*, ATII epithelial cells from newborn *Adra2a*$^{+/+}$ (*a–c*) or *Adra2a*$^{-/-}$ (*b* and *c*) mice were isolated, and marker gene expression (*a*) and morphology were determined. Low *Pecam* and *Ddr2* to *Spb* mRNA ratios in primary ATII cells as compared with intact lungs indicate low levels of contaminating fibroblasts (*Ddr2*) or endothelial cells (*Pecam*) (*a*, $n =$ 3–4 per group, *, $p < 0.05$, ***, $p < 0.001$ *versus Adra2b*$^{+/+}$ lung). Primary cultivated ATII cells of *Adra2b*$^{-/-}$ neonatal mice did not show morphological differences but express significantly more *Shh* as compared with wild-type neonatal ATII cells (*b* and *c*, $n = 5$ per genotype, **, $p < 0.01$ *versus Adra2b*$^{+/+}$; *b*, *bars*, 20 μm). *d–i*, lung sections derived from E17.5 wild-type or α_{2B}-deficient embryos were cultivated *in vitro* and stimulated with 25 nmol/liter medetomidine for 24 h. *d* and *e*, medetomidine suppressed sonic hedgehog mRNA (*d*) and protein levels (*e*) in wild-type lungs but not in *Adra2b*$^{-/-}$ tissue (*d*). *f*, lung histology did not differ between genotypes and treatment groups after *in vitro* incubation (*bars*, 10 μm; *B*, bronchi). *g* and *i*, expression of cyclin D1 (*Ccnd1*, *g*) and the SHH downstream target genes, *Gli1* and *Gli2* (*i*), was reduced by medetomidine in wild-type lungs *in vitro*. *Gli3* expression was not significantly reduced (*d–i*, $n = 6$ per group, *, $p < 0.05$, **, $p < 0.01$ *versus* untreated *Gli3*, $p = 0.052$).

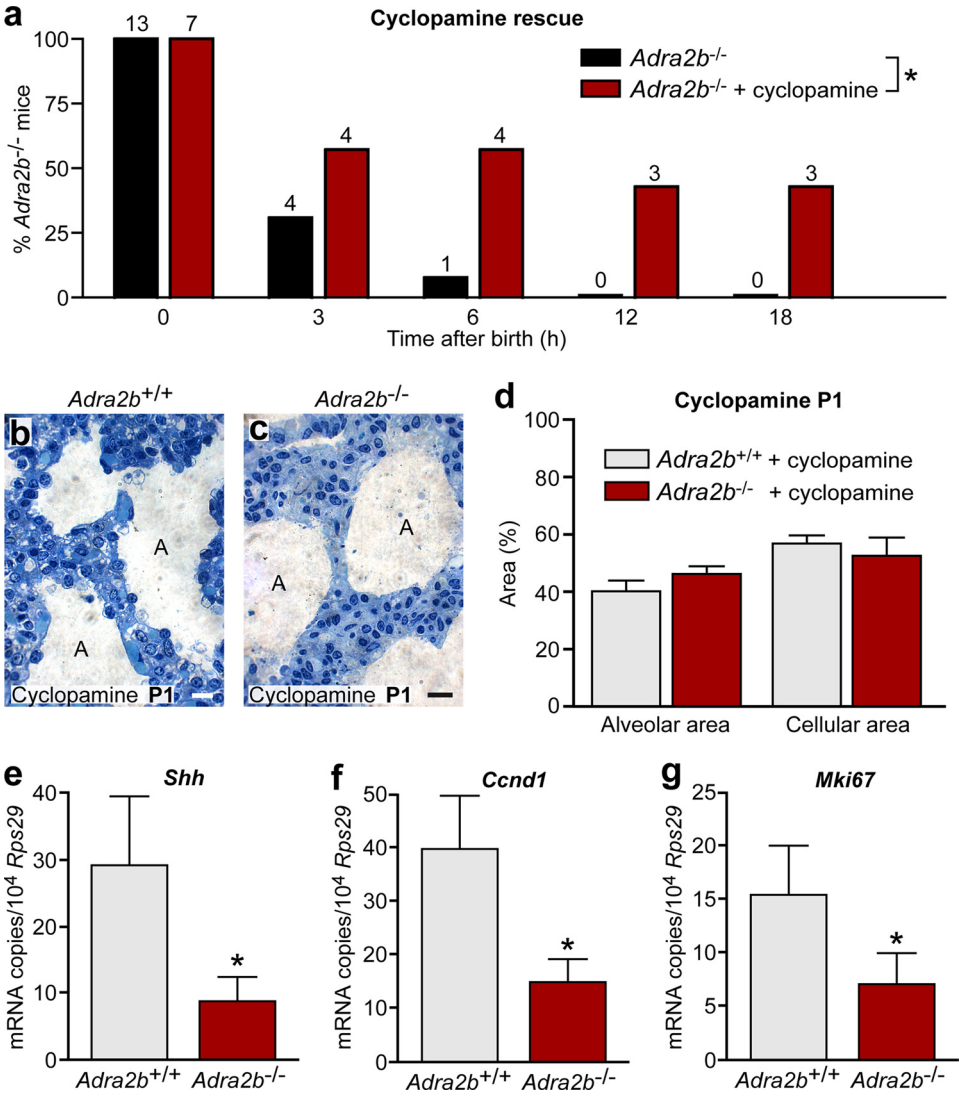

FIGURE 6. **Effect of cyclopamine treatment on perinatal lethality, lung structure, and gene expression.** *a*, pregnant females from heterozygous crosses were treated with cyclopamine starting on day E17.5 (18 mg/kg of body weight, injected subcutaneously), and survival of *Adra2b*$^{-/-}$ offspring was compared with vehicle-treated *Adra2b*$^{-/-}$ mice. *Numbers* above columns indicate living *Adra2b*$^{-/-}$ mice (*, $p = 0.017$, log rank test). *b–d*, lung histology of cyclopamine-treated wild-type and mutant mice on day P1 did not differ between genotypes (*bars*, 10 μm; A, alveoli). *e–g*, cyclopamine treatment resulted in a significant suppression of *Shh* expression and the cell cycle markers *Ccnd1* and *Mki67* in *Adra2b*$^{-/-}$ lungs as compared with wild-type lungs on day P1 ($n = 4–6$ per group, *, $p < 0.05$ *versus* *Adra2b*$^{+/+}$).

tors are also essential for perinatal survival and adaption of the lung of newborn mice.

Several factors confirm the finding that perinatal lethality in *Adra2b*$^{-/-}$ mice is caused by lung failure. Several essential postnatal functions including breathing, drinking behavior, and cardiac rhythm appeared normal in α_{2B}-deficient mice immediately after birth. Several hours after birth, mutant mice became cyanotic, which is consistent with the observed thickening of interalveolar septi and reduced air spaces in the lung. Thus, the distance for gas exchange between alveoles and capillaries may be increased in mutant mice, reducing gas exchange and ultimately causing respiratory failure. In accordance with this mechanism, hypoxia may elicit pulmonary vasoconstriction, which leads to increased vascular resistance and dilatation of the right ventricle. A similar chain of events has been observed in mice lacking pituitary adenylate cyclase-activating polypeptide type I receptors (25). In contrast to the embryonic period, where α_{2B} is essential for vascular development in the placenta, pulmonary vascular development was not significantly altered by α_{2B}-ablation as capillary density did not differ between wild-type and *Adra2b*$^{-/-}$ lungs. Other organ systems that have been suggested to be regulated by α_{2B}-receptors also developed normally in *Adra2b*$^{-/-}$ mice. α_{2B}-Receptors are abundantly expressed in hematopoietic cells in the fetal liver (26). However, erythrocyte counts and morphology as well as hemoglobin synthesis in the liver were normal as compared with wild-type mice.

Our data suggest that dysregulation of sonic hedgehog signaling in the lung is causally related to the developmental defect in the lung. SHH has previously been shown to play an important role in the determination of cell fate and embryonic patterning. General ablation of SHH expression caused severe lung malformations, hypoplasia, and deficient branching morphogenesis (27, 28). Transgenic re-expression of SHH in respiratory epithelial cells substantially rescued the peripheral lung morphogenesis defect observed in SHH-deficient mice (29). Overexpression of SHH under control of the surfactant protein C promoter led to a similar phenotype as observed in α_{2B}-deficient mice, *i.e.* early postnatal lethality, thickened septi in the lung, and proliferation of mesenchymal cells (30). Similar to the α_{2B}-deficient mice, SHH transgenic pups died soon after birth due to respiratory failure (30).

SHH may play a causal role in the phenotype elicited by ablation of α_{2B}-expression (Fig. 7). *In vitro*, stimulation of α_{2B}-receptors suppressed expression of SHH and cell cycle markers cyclin D1 and Ki67. These results indicate that SHH is under tonic inhibition by α_{2B}-adrenoceptors *in vivo*. Furthermore, inhibition of enhanced SHH signaling by the smoothened antagonist cyclopamine partially rescued the perinatal lethality and the pulmonary phenotype. Cyclopamine has been demonstrated to specifically block hedgehog signaling by direct binding to the G protein-coupled receptor smoothened (20, 21). Further studies are required to identify the precise intracellular pathway linking α_{2B}-receptors with SHH repression. In addition, it will be important to define the role of α_{2B}-receptors in the development of pulmonary diseases, including lung fibrosis

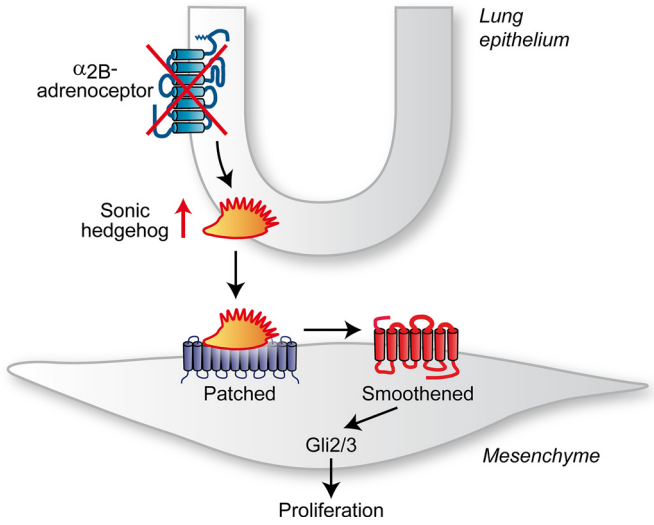

FIGURE 7. **Model of the role of α_{2B}-adrenoceptors in perinatal lung development.** α_{2B}-Adrenoceptors are expressed in ATII epithelial cells where they suppress expression of SHH. Ablation of α_{2B} expression in mice leads to enhanced levels of SHH, which stimulates mesenchyme proliferation in interalveolar septi, thus resulting in perinatal respiratory failure.

and inflammation. In conclusion, α_{2B}-adrenoceptors suppress sonic hedgehog to control this important epithelial-mesenchymal signaling pathway, which is essential for lung development.

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