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Ionotropic GABA receptor expression in the lung during development

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ABSTRACT

Cl^- transport is essential for lung development. Because gamma-aminobutyric acid (GABA) receptors allow the flow of negatively-charged Cl^- ions across the cell membrane, we hypothesized that the expression of ionotropic GABA receptors are regulated in the lungs during development. We identified 17 GABA receptor subunits in the lungs by real-time PCR. These subunits were categorized into four groups: Group 1 had high mRNA expression during fetal stages and low in adults; Group 2 had steady expression to adult stages with a slight up-regulation at birth; Group 3 showed an increasing expression from fetal to adult lungs; and Group 4 displayed irregular mRNA fluctuations. The protein levels of selected subunits were also determined by Western blots and some subunits had protein levels that corresponded to mRNA levels. Further studied subunits were primarily localized in epithelial cells in the developing lung with differential mRNA expression between isolated cells and whole lung tissues. Our results add to the knowledge of GABA receptor expression in the lung during development.

1. Results and discussion

1.1. Significance

Cl^- channels play an important role in the lung throughout an animal's life. During the fetal stages, the lung is a fluid-secreting organ. The main driving force for fluid secretion is the active secretion of Cl^- into the developing lungs. Therefore, Cl^- channels are indispensable for fetal lung development and morphogenesis (Folkesson et al., 1998; Olver et al., 2004; Bland and Nielson, 1992). On the other hand, the adult lung is air-filled and maintains a "dry" state. The apical surface is covered by a thin layer of alveolar surface fluid. This layer of fluid is required for the proper air exchange and airway defenses. Cl^- channels are also essential for maintaining the composition and volume of the alveolar surface fluid (Brochiero et al., 2004).

Several Cl^- channels have previously been studied in the lung. The cystic fibrosis transmembrane conductance regulator (CFTR) (Harris et al., 1991), the voltage-gated Cl^- channels, ClC2 (Thiemann et al., 1992), ClC3 (Lamb et al., 2001), and ClC5 (Edmonds et al., 2002), and Na^+–K^+–Cl^- cotransporter (NKCC) (Rochelle et al., 2000) have been detected in fetal lungs, distal airway epithelial cells and alveolar epithelial cells. Some of the channels have been shown to be involved in fluid homeostasis in fetal and adult lungs (Brochiero et al., 2004; Blaisdell et al., 2000; Fang et al., 2002). These Cl^- channels appear in early gestation and are expressed relatively high throughout the fetal stage, but decrease at late gestation and remain low during adult stages (Harris et al., 1991; Murray et al., 1996, 1995; Lamb et al., 2001). The Cl^- channels may participate in fluid secretion in fetal lungs since the lung undergoes a change from fluid secretion to absorption at birth. However, CFTR and NKCC knock-out mice were not fatal (Gillie et al., 2001; Snouwaert et al., 1992). In these mice, other Cl^- channels may compensate for those channels.

Ionotropic γ-aminobutyric acid (GABA) receptors are the most important Cl^- channels in the central nervous system (CNS), and their expression has also been found in peripheral organs. These receptors mediate a fast inhibitory neurotransmission in the CNS but their functions in peripheral organs have not been extensively studied (Akinci and Schofield, 1999). Ionotropic GABA receptors can be categorized into $GABA_A$ and $GABA_C$ receptors based on their subunit compositions and pharmacological properties (Bormann, 2000). Nineteen GABA receptor subunits have been cloned from rats, which include α1–6, β1–3, γ1–3, ρ1–3, δ, θ, ε, and π (Whiting et al., 1999). We have recently identified the $GABA_A$ receptor π subunit as a novel alveolar epithelial type II cell marker (Chen et al., 2004b). We have also detected the α1, α3, β2, γ2, and γ3 subunits in adult type II cells (Jin et al., 2005). Furthermore, we have provided evidence that GABA receptors contribute to fluid balance in adult lungs (Jin et al., 2006).

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A functional $GABA_A$ or $GABA_C$ receptor requires five subunits (Whiting et al., 1999). While $GABA_A$ receptors are assembled from at least 3 different subfamilies of subunits, $GABA_C$ receptors can be formed from only the ρ subunit family. The most common combination of a $GABA_A$ receptor is αβγ, in which α and β subunits appear to be essential and the γ subunit can be replaced by other subunits (Sieghart et al., 1999). In addition to those common subunits, "rare" subunits such as θ, ε, δ, and π can assemble with αβγ (Sieghart and Sperk, 2002; Sieghart et al., 1999). For example, the π subunit was found to assemble with α1β3γ2 or α5β3γ3 in the co-transfected cell lines (Hedblom and Kirkness, 1997; Neelands and Macdonald, 1999).

In this study, we investigated the developmental regulation of all the ionotropic GABA receptor subunits in the lung and pulmonary epithelial cells. This is the first study on the dynamic changes of GABA receptor subunits in developing lungs.

1.2. Quantification of the mRNA levels of GABA receptor subunits in fetal rat lungs and pulmonary epithelial cells

By using real-time PCR, we determined the mRNA abundance of 19 GABA receptor subunits (α1–6, β1–3, γ1–3, δ, θ, ε, π, and ρ1–3) in fetal lungs and fetal pulmonary epithelial cells on gestational day 18 (D18), which was selected to represent the late pseudoglandular stage of fetal lung development. Brain tissue from adult rats was used as a positive control and contained more than 50 copies per 10^8 copies of 18S rRNA for all the subunits (Fig. 1). The α1 subunit had the highest expression in brain compared with other subunits (>10^5 copies per 10^8 copies of 18S rRNA). Brain contained 10^4–10^5 copies per 10^8 copies of 18S rRNA of α2–6, β1–3, γ1–3, δ, ε, ρ1, and ρ3 subunits. In the lung, α5, α6, ε, π, ρ1, and ρ3 subunits had >100 copies per 10^8 copies of 18S rRNA. The copy numbers for α1, α2, α3, α4, β2, γ3, and ρ2 subunits in the lung were 10–100 copies per 10^8 copies of 18S rRNA. In pulmonary epithelial cells, α1, α3, α4, β2, π, and ρ1–3 subunits had >100 copies per 10^8 copies of 18S rRNA. Some of those subunits (α1, α3, and α4) appear to be enriched in pulmonary epithelial cells in comparison with lung tissue. As a whole, the mRNA levels of GABA receptor subunits in the lung and pulmonary epithelial cells were much lower than that in the brain, especially for α, β, and γ subunit families. However, π, ρ1, ρ2, and ρ3 subunits had a similar or higher abundance of mRNA in lungs in comparison with that in brains (Fig. 1).

1.3. Developmental alteration of GABA receptor mRNA in rat lungs

The 17 subunits expressed in the lung can be classified into four groups based on the similarity of changes in expression during lung development (Fig. 2A–D). Group 1 was composed of 6 subunits (α2, α5, α6, γ3, β1, and ρ3). The genes in this group exhibited a decreasing trend from D18 to adult. These subunits had significantly lower mRNA expression in adult lungs in comparison with the fetal or newborn lungs (Fig. 2A). Group 2 had 5 subunits (α3, γ1, γ2, θ, and ρ1). The mRNA expression in this group was relatively steady from fetal to adult lungs, but was slightly up-regulated at birth (Fig. 2B). Group 3 included 3 subunits (α1, β2, and π). The genes in this group showed an increasing trend from fetal D18 to adult (Fig. 2C). Group 4 was composed of 3 subunits (α4, ε, and ρ2). The genes in this group exhibited irregular fluctuation and had no significant difference among all the time points (Fig. 2D).

1.4. Developmental changes of GABA receptor protein during lung development

We further determined the changes in protein levels of GABA receptor subunits during lung development. We chose one subunit in each group as a representative. Whole lung tissue was run on SDS–PAGE, and Western blot was performed using specific antibodies. As shown in Fig. 3, the protein level of the β1 subunit in Group 1 was high at D16 and D19 and declined after D21. The γ2 subunit (Group 2) protein levels were the same at D16, D19, and D21, and then dropped after birth. On the other hand, the π subunit (Group 3) protein level was the highest in the adult lungs. Finally, the α4 subunit (Group 4) expressed the greatest amount of protein at D16, followed by a gradual decline in expression. The equal protein loading was confirmed by using anti-β-actin antibodies (Fig. 3).

1.5. Cellular localization of GABA receptors in fetal lung tissue

Using immunostaining, we analyzed the location of GABA receptors in D20 and new born (NB) lung tissues. The π subunit showed positive staining primarily in the airway epithelial cells in both D20 and NB lung tissues in particular at D20 (Fig. 4A insert). The π subunit also co-localized partially with SP-C, an alveolar type II cell marker (Fig. 4B). The β1 subunit had a similar

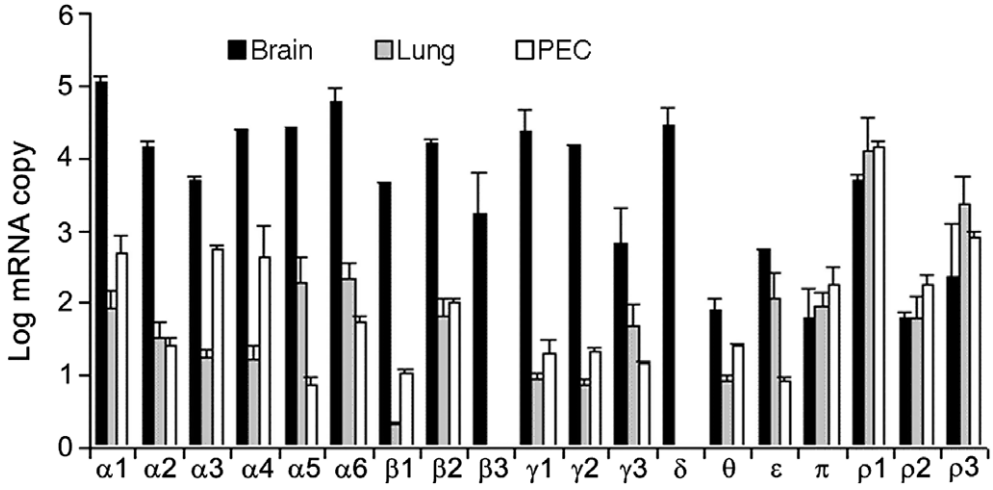

Fig. 1. Expression of GABA receptor subunits in fetal rat lungs and pulmonary epithelial cells. Total RNA was isolated from rat fetal lungs and pulmonary epithelial cells (PEC) at gestational day 18 and reverse-transcribed into cDNA. The mRNA abundance of 19 ionotropic GABA receptors was quantified by real-time PCR, normalized to 18S rRNA and expressed as log copy number per 10^8 copies of 18S rRNA. The adult brain was used as a positive control. Data shown are means ± SE ($n \geqslant 3$ independent cell preparations).