

REVIEW

γ -aminobutyric acid nurtures allergic asthma

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Clinical & Experimental Allergy

Summary

Asthma often occurs as a result of immune-based inflammatory responses, which consequently cause pathological changes in airway structural cells. However, the underlying mechanisms of airway pathology in asthma are still not fully understood. Our recent studies revealed a critical role of γ -aminobutyric acid (GABA) signalling pathway in the airway epithelium of allergic asthma through its ability to stimulate mucus production. This review briefly describes the GABAergic signalling system and its role in the regulation of mucus protein production in bronchial airway epithelial cells.

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Asthmatic reactions in bronchial airway epithelium

Despite recent advances in our understanding of the pathologic basis of asthma, there are still major gaps in our understanding of the functional significance of much of the underlying pathology. In general it is appreciated that dysfunction and symptoms in asthma may occur as a result of immune-based inflammatory responses (often triggered by allergens), or as a result of pathological changes occurring in airway structural cells, often collectively grouped under the term airway remodelling. Included under this heading are changes in airway epithelial cells (AECs), subepithelial fibroblasts, and airway smooth muscle cells. AECs likely play a key role in airway remodelling, particularly in the bronchial airways, and in this review, we will focus upon the asthmatic pathology of bronchial airway epithelium.

The bronchial segment of airway epithelium consists of ciliated cuboidal cells and non-ciliated Clara cells [1, 2]. These AECs protect the subepithelial tissue from airborne irritants not only by forming a physical barrier, but also by enhancing mucociliary clearance. Mucus is a complex aqueous solution containing glycoproteins (mainly

mucins) that lines the bronchial lumen above a thin liquid sheet of extracellular solution. Mucins are produced and then stored in periodic-acid-Schiff -positive secretory granules in mucous cells of the submucosal glands. When secreted into the airway lumen, mucins develop into the mucus that moisturizes and lubricates mucosal surfaces thereby protecting AECs through trapping of inhaled toxic particles and bacteria. The mucous layer is continually removed from the epithelial surface by the actions of ciliary beating. The maintenance of normal mucociliary function largely depends on the appropriate volume of the liquid sheet, which is controlled by water movements across the apical membrane of AECs. Specifically, the level of the liquid layer is primarily controlled by coordinated Cl^- secretion and Na^+ absorption, which occurs at the apical membrane of AECs. It was believed that Cl^- secretion is mediated primarily by a Ca^{2+} -activated Cl^- channels [3] and by the cystic fibrosis transmembrane regulator (CFTR) [3], whereas Na^+ absorption is carried out by the epithelial Na^+ channel [4]. In airway diseases, the balance is shifted from water secretion to mucus secretion. For example, in cystic fibrosis patients, the liquid level is decreased, increasing mucus viscosity, reducing mucociliary clearance and eventually leading to an accumulation of bronchial mucus, frequently with a concurrent infection [5, 6].

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The term 'airway remodelling' commonly refers to structural changes that occur in conjunction with, or because of, chronic airway inflammation [7], such as that seen in asthma or chronic obstructive pulmonary disease [8]. These structural alterations in AECs are characterized by goblet cell (GC) hyperplasia [9, 10], which includes cell proliferation and phenotypic transdifferentiation. In human asthma, GC at distal airways are derived from Clara cells [11] as seems to be the case in the mouse lung [12]. The consequences of epithelial remodelling also include incompletely reversible airway oedema and mucous hypersecretion.

GC hyperplasia and mucous hypersecretion with complete airway occlusion has been observed in some cases of fatal asthma [13]. The mechanism of GC hyperplasia remains unclear. It is known, however, that the classic T-helper type 2 (TH₂) cytokine IL-13 can activate phosphoinositide-3 kinase (PI3K) and its downstream effector Akt (protein kinase B) [14], leading to airway remodelling including GC hyperplasia in animal models of asthma [15–17]. This cytokine action may involve transactivation of epidermal growth factor receptors [18]. Notably, recent reports showed that Th2 cytokines stimulated a non-CFTR Cl[−] conductance [19] thereby switching AECs to a secretory phenotype [19, 20]. These studies suggest that epithelial Cl[−] conductance plays an important role in GC hyperplasia and mucus overproduction.

γ-aminobutyric acidergic signalling

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS)

[21]. Knowledge of GABA signalling is mainly from studies on CNS neurons. As illustrated in Fig. 1, GABA is synthesized from glutamic acid (or glutamate) by glutamic acid decarboxylase (GAD). There are two forms of GAD, GAD65 and GAD67 [22]. In neurons GAD65 is primarily anchored to synaptic vesicles in nerve terminals while GAD67 is present mainly in the cytosol of the cell body [23]. The GABA molecules stored in the vesicles are released to the extracellular space via exocytosis, whereas mechanisms for cytosol GABA release remains uncertain. Expression of GAD67 predominates during early development or after injury suggesting that it might have a role in producing GABA for trophic signalling [22, 24].

GABA activates two subfamilies of cell surface receptors: GABA_AR and GABA_BR. GABA_ARs are pentameric anionic (Cl[−]) channels [25], while GABA_BRs are G-protein-coupled receptors. In this short review we will primarily focus on the potential role of GABA_AR-mediated signalling in asthma.

Subtypes of GABA_ARs differ in their affinity for ligands, channel kinetics and subcellular localization [26] as a consequence of differing possible combinations of 19 subunits (α1–6, β1–3, γ1–3, ρ1–3, δ, θ, ε, and π) [27]. GABA_AR-mediated anionic conductance can be effectively blocked by the competitive receptor antagonist bicuculline or by the noncompetitive channel blocker picrotoxin.

The trafficking and cell surface targeting of GABA_ARs are regulated by PI3K, which can be activated by diverse signals including insulin [28, 29], growth factors [30–34], and IL [35]. In this regard, it is reported that insulin can

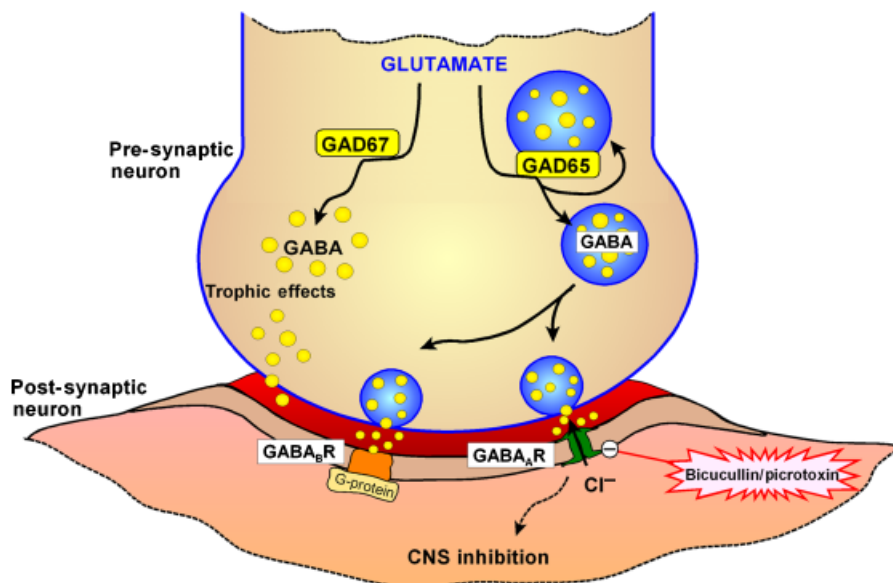


Fig. 1. γ-aminobutyric acid (GABA) signalling in the central nervous system (CNS). Decarboxylation of glutamate by GAD67 results in production of cytosolic GABA, while decarboxylation by vesicle membrane bound GAD65, increases vesicular GABA. Once released into the synaptic cleft, GABA activates GABA_A or GABA_B receptors. Activation of GABA_A receptors, which are chloride channels, typically results in suppression of CNS neuronal activity.

rapidly recruit GABA_ARs to the surface of neuronal cells [36, 37], and PI3K is associated with the regulation of β_2 subunit of GABA_ARs ($\beta_{2\text{GABAR}}$) [38]. Upon stimulation with insulin, PI3K stimulates phosphorylation of Akt, which in turn phosphorylates $\beta_{2\text{GABAR}}$ [36, 37], resulting in a translocation of $\beta_{2\text{GABAR}}$ to the plasma membrane [37, 38]. IL-1 β also activates PI3K/Akt signalling in the neurons and facilitates targeting of GABA_ARs to the surface [35].

GABA_ARs mediate two forms of inhibition in central neurons. At the synapse the GABAergic pre-synaptic terminal releases large amount of GABA via vesicular exocytosis following action potential. In response to the pulsatile release of GABA, the GABA_ARs at the post-synaptic domain generate a peak ('phasic') current, which is important for generating patterned activities in neuronal networks [39, 40]. Importantly, extrasynaptic GABA_ARs were persistently activated by ambient levels of GABA, generating a 'tonic' current [39, 40–43]. The ambient GABA is likely released from the cytosolic pool of adjacent GABAergic cells. This tonic GABAergic activity may provide a trophic signal in both neurons [44] and other cell types [45].

GABA_AR activation inhibits the majority of mature neurons but paradoxically excites embryonic neurons. Whether inhibition or excitation of neurons occurs depends upon the transmembrane gradient of Cl[−]. During embryonic development GABA is excitatory and its receptors lead to increased cytosolic Ca²⁺ levels a signal that directs the migration of post-mitotic neuroblasts and stimulates their motility [46]. It is believed that GABA acts as a diffusible trophic factor and that GABA_ARs function as its sensors. GABA receptors in the embryonic brain are critical for regulation of cell proliferation, differentiation [47], growth [48], and migration [39].

Association of γ -aminobutyric acidergic signalling with asthmatic reactions in airway epithelial cell

Studies have shown that GABA is also synthesized in non-neuronal cells and may also act as a trophic factor [45]. A previous study reported that mRNAs encoding for GABA_AR subunits are expressed in type II alveolar ECs [49]. This report raised interesting and critical questions: Are GABA_AR subunit proteins expressed in AECs? If so, what is the source of GABA for activation of the receptors? Most importantly, what could be the functions of GABA_ARs in the lung? Bearing these questions in mind, we carried out serial experiments to explore the potential physiological role and/or pathophysiological consequence of GABA_ARs in the lung [50].

Our immunoblotting assays revealed that GAD65/67 as well as several GABA_AR subunits were expressed in the lung cells of BALB/c mice, in primary human small AECs (SAECs) [51, 52], in human AEC line BEAS-2B cells and in human type II alveolar A549 ECs, respectively. Immuno-

fluorescent staining further showed that GAD was expressed in all AECs in naïve mice. In contrast, GABA_AR β -subunits were expressed only on the apical membrane of a small portion of AECs. Consistent with this observation, perforated patch-clamp recordings, which do not alter the concentration of intracellular Cl[−], show that GABA evoked rapid inward currents in some SAECs. The GABA-evoked current is effectively blocked by the GABA_AR antagonist picrotoxin or bicuculline, confirming that some lung ECs possess functional GABA_AR channels. Notably, we found that the reversal potential of the GABA-current (E_{GABA}) in A549 cells was about −12 mV and the 'resting' membrane potential (E_R) of the cells ranges from −33 to −53 mV. Application of GABA therefore induced a depolarization in these lung ECs, indicative of GABA-induced Cl[−] efflux from the cells. Furthermore, applications of picrotoxin induced hyperpolarizations and generated small outward currents, demonstrating that GABA_ARs were persistently activated. These combined data indicate that an 'excitatory', rather than an 'inhibitory', GABAergic activity persistently exists in the lung ECs.

Cl[−] transport is critical for EC proliferation, electrolyte–water transport and, hence mucus overproduction [19, 20]. Our studies also discovered that treating SAECs with GABA (10 μ mol/L) increased BrdU incorporation and greatly enhanced intra- and extra-cellular alcian blue staining (note: alcian blue stains glycoproteins, such as mucins [53]). These stimulating effects of GABA were largely prevented by picrotoxin. These results strongly suggested that GABA_AR activation stimulated cell proliferation and mucus production. Airway GC hyperplasia and mucus hypersecretion often occur in severe asthma [9, 10]. Indeed, in BALB/c mice sensitized and challenged with ovalbumin (OVA) [54, 55], the levels of cytosolic GAD expression and the expression of GABA_AR β -subunits in the apical membrane were greatly increased in AECs. Furthermore, the increased expression of GAD and GABA_ARs is associated with GC hyperplasia and mucus overproduction. Immunoblots of the lung tissues confirmed the increase in GAD and GABA_ARs. These results indicate that the AEC GABAergic system is up-regulated in allergic asthmatic reactions. Of note, in our study GABA_AR subunits were not stained in airway smooth muscle cells of naïve or OVA-treated mice, implying that the GABA signalling is selectively associated with epithelial cell functions.

As aforementioned, IL-13 is a critical regulator in animal models of allergic asthma [15, 56, 57]. This cytokine may mediate the up-regulation of the GABAergic signalling in modelled asthmatic reactions. Indeed, the levels of IL-13 were greatly increased in bronchoalveolar lavage fluid (BALF) of the OVA-sensitized and challenged mice. Furthermore, treatment of SAECs with IL-13 for 6 days increased the expression of GAD and of $\beta_{2\text{GABAR}}$. Moreover, intranasal instillation of IL-13 enhances the levels of GAD and GABA_ARs in AECs *in vivo*. These data suggest

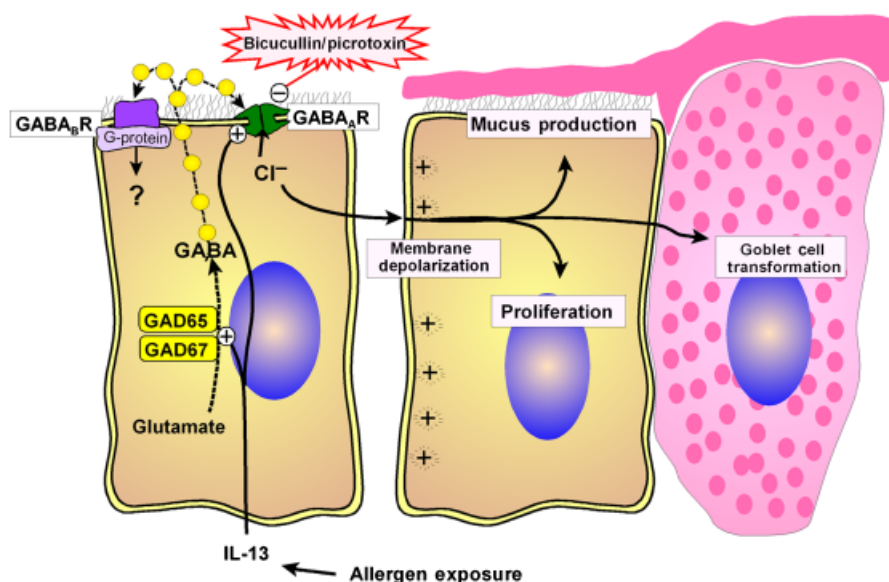


Fig. 2. γ -aminobutyric acid (GABA) involvement in epithelial biology of asthma. Increase in IL-13, either directly, or secondary to allergen exposure, results in increased expression of GAD65/67 and GABA_A receptors in airway epithelial cells (AECs). The enhanced autocrine GABA signalling results in chloride efflux, depolarization, cell proliferation, and mucus hypersecretion of AECs. Selective GABA_A receptor antagonism, with bicuculline or picrotoxin prevents allergen, or IL-13 induced goblet cell transformation. GABA_BR has also been identified on AECs, but its involvement in AEC biological/pathological events remains largely unknown.

that during the development of allergen-induced airway responses, the AEC GABAergic system is activated by IL-13-initiated signals. To block the GABA signalling, we administered the GABA_AR antagonists picrotoxin or bicuculline intraperitoneally or intranasally to OVA- or IL-13-treated mice. The GABAergic blockade greatly suppressed the GC hyperplasia as well as the mucus overproduction induced by OVA and/or by IL-13. In contrast, inhibiting GABA_ARs did not block the OVA-induced IL-13, nor affect inflammatory cell infiltration in the airway wall or in BALF. These results suggest that in AECs the up-regulated GABAergic signalling is causally involved in some of the asthmatic-related reactions. Our findings also demonstrated that the GABAergic activity was downstream of IL-13 signalling. In relation to this notion, it is known that IL-13 can activate PI3K/Akt in epithelial cells [14] and that activation of PI3K/Akt causes targeting of GABA_ARs to the post-synaptic domain of neuronal cells. It is plausible that IL-13 up-regulates the epithelial GABA signalling through activation of PI3K/Akt. Figure 2 depicts proposed mechanisms of GABA_AR-mediated asthmatic reactions in AECs.

Recent studies have shown that GABA_BRs were also expressed in mucin-secreting and ciliated columnar AECs [58] and airway smooth muscle cells [59], and activation of GABA_BRs inhibited adenylyl cyclase activity through G(i) protein coupling in these airway cells [58, 59]. The role of GABA_BR-mediated signalling in airway asthmatic reaction awaits investigation.

Our discovery of the GABA_AR-mediated large Cl⁻ conductance in AECs is of considerable significance because it is fundamentally different from previously described types of Cl⁻ channels in these cells. Importantly, our data indicated that the GABAergic activity in AECs was causally associated with GC hyperplasia and mucus overproduction, and that the expression levels of GABAergic signalling molecules in AECs were closely correlated to the degree of the allergy-induced airway hyperresponsiveness in different mouse strains. Mucus overproduction is a primary cause of death in severe asthma. Remarkably, GABA_ARs are expressed on the apical membrane of AECs; hence, their activity can be readily modulated by inhalation of selective agonists and/or antagonists of the receptor. Indeed, inhibition of the pulmonary GABAergic system blocks airway GC hyperplasia and mucus overproduction. The significance of our study was highly evaluated by a commentary in *Nature Medicine* [60]. Further studies on the mechanisms by which the GABAergic system regulates AEC proliferation and metaplasia will not only deepen our current understanding of airway epithelial remodelling, but may also lead to new therapeutic strategies for management of airway hypersecretion in asthma.

References

- 1 Davies DE. The bronchial epithelium: translating gene and environment interactions in asthma. *Curr Opin Allergy Clin Immunol* 2001; 1:67–71.

- 2 Matthay MA, Robriquet L, Fang X. Alveolar epithelium: role in lung fluid balance and acute lung injury. *Proc Am Thorac Soc* 2005; 2:206–13.
- 3 Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. *Physiol Rev* 2002; 82:503–68.
- 4 Garty H, Palmer LG. Epithelial sodium channels: function, structure, and regulation. *Physiol Rev* 1997; 77:359–96.
- 5 Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. *Adv Drug Deliv Rev* 2002; 54:1359–71.
- 6 Boucher RC. Regulation of airway surface liquid volume by human airway epithelia. *Pflugers Arch* 2003; 445:495–8.
- 7 Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. *Curr Opin Pulm Med* 2000; 6:15–20.
- 8 Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001; 164:S28–38.
- 9 Rogers DF. Airway goblet cell hyperplasia in asthma: hypersecretory and anti-inflammatory? *Clin Exp Allergy* 2002; 32:1124–7.
- 10 Rogers DF. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr Opin Pharmacol* 2004; 4:241–50.
- 11 Kilburn KH. Functional morphology of the distal lung. *Int Rev Cytol* 1974; 37:153–270.
- 12 Hayashi T, Ishii A, Nakai S, Hasegawa K. Ultrastructure of goblet-cell metaplasia from Clara cell in the allergic asthmatic airway inflammation in a mouse model of asthma in vivo. *Virchows Arch* 2004; 444:66–73.
- 13 Kuyper LM, Pare PD, Hogg JC *et al.* Characterization of airway plugging in fatal asthma. *Am J Med* 2003; 115:6–11.
- 14 Atherton HC, Jones G, Danahay H. IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation. *Am J Physiol Lung Cell Mol Physiol* 2003; 285:L730–9.
- 15 Grunig G, Warnock M, Wakil AE *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282:2261–3.
- 16 Laoukili J, Perret E, Willems T *et al.* IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J Clin Invest* 2001; 108:1817–24.
- 17 Tyner JW, Kim EY, Ide K *et al.* Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. *J Clin Invest* 2006; 116:309–21.
- 18 Nadel JA. Role of epidermal growth factor receptor activation in regulating mucin synthesis. *Respir Res* 2001; 2:85–9.
- 19 Danahay H, Atherton H, Jones G, Bridges RJ, Poll CT. Interleukin-13 induces a hypersecretory ion transport phenotype in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L226–36.
- 20 Danahay H, Atherton HC, Jackson AD, Kreindler JL, Poll CT, Bridges RJ. Membrane capacitance and conductance changes parallel mucin secretion in the human airway epithelium. *Am J Physiol Lung Cell Mol Physiol* 2006; 290:L558–69.
- 21 Olsen RW, Tobin AJ. Molecular biology of GABAA receptors. *FASEB J* 1990; 4:1469–80.
- 22 Martin DL, Rimvall K. Regulation of gamma-aminobutyric acid synthesis in the brain. *J Neurochem* 1993; 60:395–407.
- 23 Martin DL, Barke KE. Are GAD65 and GAD67 associated with specific pools of GABA in brain? *Perspect Dev Neurobiol* 1998; 5:119–29.
- 24 Belhage B, Hansen GH, Elster L, Schousboe A. Effects of gamma-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect Dev Neurobiol* 1998; 5:235–46.
- 25 Moss SJ, Gorrie GH, Amato A, Smart TG. Modulation of GABAA receptors by tyrosine phosphorylation. *Nature* 1995; 377:344–8.
- 26 Luscher B, Keller CA. Regulation of GABAA receptor trafficking, channel activity, and functional plasticity of inhibitory synapses. *Pharmacol Ther* 2004; 102:195–221.
- 27 Smith TA. Type A gamma-aminobutyric acid (GABAA) receptor subunits and benzodiazepine binding: significance to clinical syndromes and their treatment. *Br J Biomed Sci* 2001; 58:111–21.
- 28 Kim SO, Hasham MI, Katz S, Pelech SL. Insulin-regulated protein kinases during postnatal development of rat heart. *J Cell Biochem* 1998; 71:328–39.
- 29 Ueki K, Yballe CM, Brachmann SM *et al.* Increased insulin sensitivity in mice lacking p85beta subunit of phosphoinositide 3-kinase. *Proc Natl Acad Sci USA* 2002; 99:419–24.
- 30 Lin SY, Cui H, Yusta B, Belsham DD. IGF-I signaling prevents dehydroepiandrosterone (DHEA)-induced apoptosis in hypothalamic neurons. *Mol Cell Endocrinol* 2004; 214:127–35.
- 31 Jiang Q, Zhou C, Bi Z, Wan Y. EGF-induced cell migration is mediated by ERK and PI3K/AKT pathways in cultured human lens epithelial cells. *J Ocul Pharmacol Ther* 2006; 22:93–102.
- 32 Kiyatkin A, Aksamitiene E, Markevich NI, Borisov NM, Hoek JB, Kholodenko BN. Scaffolding protein Grb2-associated binder 1 sustains epidermal growth factor-induced mitogenic and survival signaling by multiple positive feedback loops. *J Biol Chem* 2006; 281:19925–38.
- 33 Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *J Neurosci* 2005; 25:11288–99.
- 34 Volosin M, Song W, Almeida RD, Kaplan DR, Hempstead BL, Friedman WJ. Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. *J Neurosci* 2006; 19:7756–66.
- 35 Serantes R, Arnalich F, Figueroa M *et al.* Interleukin-1beta enhances GABAA receptor cell-surface expression by a phosphatidylinositol 3-kinase/Akt pathway: relevance to sepsis-associated encephalopathy. *J Biol Chem* 2006; 281:14632–43.
- 36 Wan Q, Xiong ZG, Man HY *et al.* Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature* 1997; 388:686–90.
- 37 Wang Q, Liu L, Pei L *et al.* Control of synaptic strength, a novel function of Akt. *Neuron* 2003; 19:915–28.
- 38 Vetiska SM, Ahmadian G, Ju W, Liu L, Wymann MP, Wang YT. GABA(A) receptor-associated phosphoinositide 3-kinase is required for insulin-induced recruitment of postsynaptic GABA(A) receptors. *Neuropharmacology* 2007; 52:146–55.
- 39 Mody I. Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. *Neurochem Res* 2001; 26:907–13.
- 40 Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active GABAA receptors: modulating gain and maintaining the tone. *Trends Neurosci* 2004; 27:262–9.
- 41 Semyanov A, Walker MC, Kullmann DM. GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat Neurosci* 2003; 6:484–90.
- 42 Cope DW, Hughes SW, Crunelli V. GABAA receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci* 2005; 25:11553–63.

- 43 Caraiscos VB, Elliott EM, You-Ten KE *et al.* Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA* 2004; 101:3662–7.
- 44 Represa A, Ben-Ari Y. Trophic actions of GABA on neuronal development. *Trends Neurosci* 2005; 28:278–83.
- 45 Gladkevich A, Korf J, Hakobyan VP, Melkonyan KV. The peripheral GABAergic system as a target in endocrine disorders. *Auton Neurosci* 2006; 124:1–8.
- 46 Behar TN, Li YX, Tran HT *et al.* GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms. *J Neurosci* 1996; 16:1808–18.
- 47 Belhage B, Hansen GH, Elster L, Schousboe A. Effects of gamma-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect Dev Neurobiol* 1998; 5:235–46.
- 48 Maric D, Liu QY, Maric I *et al.* GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl[−] channels. *J Neurosci* 2001; 21:2343–60.
- 49 Chen Z, Jin N, Narasaraaju T *et al.* Identification of two novel markers for alveolar epithelial type I and II cells. *Biochem Biophys Res Commun* 2004; 319:774–80.
- 50 Xiang YY, Wang S, Liu M *et al.* A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nat Med* 2007; 13:862–7.
- 51 Hocking DC, Chang CH. Fibronectin matrix polymerization regulates small airway epithelial cell migration. *Am J Physiol Lung Cell Mol Physiol* 2003; 285:L169–79.
- 52 Baldys A, Aust AE. Role of iron in inactivation of epidermal growth factor receptor after asbestos treatment of human lung and pleural target cells. *Am J Respir Cell Mol Biol* 2005; 32:436–42.
- 53 Takeyama K, Dabbagh K, Lee HM *et al.* Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci USA* 1999; 96:3081–6.
- 54 Singer M, Martin LD, Vargaftig BB *et al.* A MARCKS-related peptide blocks mucus hypersecretion in a mouse model of asthma. *Nat Med* 2004; 10:193–6.
- 55 Seki Y, Inoue H, Nagata N *et al.* SOCS-3 regulates onset and maintenance of T(H)2-mediated allergic responses. *Nat Med* 2003; 9:1047–54.
- 56 Wynn TA. IL-13 effector functions. *Annu Rev Immunol* 2003; 21:425–56.
- 57 Kuperman DA, Huang X, Koth LL *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyper-reactivity and mucus overproduction in asthma. *Nat Med* 2002; 8:885–9.
- 58 Mizuta K, Osawa Y, Mizuta F, Xu D, Emala CW. Functional expression of GABAB receptors in airway epithelium. *Am J Respir Cell Mol Biol* 2008; 39:296–304.
- 59 Osawa Y, Xu D, Sternberg D *et al.* Functional expression of the GABAB receptor in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2006; 291:L923–31.
- 60 Corry DB, Kheradmand F. A new link to airway obstruction in asthma. *Nat Med* 2007; 13:777–8.