Check for updates

EDITORIALS

Anxiolytics for Bronchodilation: Refinements to GABA_A Agonists for Asthma Relief

In 1961, Julius Axelrod and colleagues (1) described the effects of psychotropic drugs on the uptake of catecholamine neurotransmitters by several tissues, including at the nerve endings (2), that opened the floodgates of investigation into the identity of the [uptake] channels. Such concerted efforts led to the cloning of many transporter genes, including that for neurotransmitter γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system (3).

Subsequently, it became clear that GABA exerts its physiological actions by activating the ligand-gated ionotropic Cl^- channels, GABA_A, or the metabotropic GABA_B receptor (4). GABA_A activation typically results in membrane hyperpolarization, resulting in the rapid synaptic inhibition of excitatory signals associated with anxiety disorders. Allosteric modulation of GABA_A can be achieved by a diverse class of compounds, including barbiturates, alcohol, steroids, and benzodiazepines. Benzodiazepines are of particular interest for general anxiety disorders, owing to an efficacious anxiolytic profile. Most benzodiazepines also promote muscle relaxation, which led to the subsequent discovery of GABA and associated channels and receptors in peripheral tissues (5), including airway smooth muscle (ASM) (6, 7).

Research by Charles Emala and colleagues over the past 2 decades has advanced our understanding of the physiological function of GABA channels and receptors on ASM, especially GABA_A. Activation of GABA_A channels results in ASM relaxation (6, 8, 9) and augments β_2-adrenoceptor–mediated airway relaxation (10). Mechanistically, GABA_A activation on ASM cells inhibits agonist-evoked membrane depolarization and intracellular calcium flux (8, 9). In contrast, the metabotropic GABA_B receptors are G protein–coupled receptors (GPCRs) that, on stimulation with agonists such as baclofen, inhibit (by means of G_i protein) adenylyl cyclases (11). Inhibition of adenylyl cyclases reduces the accumulation of cAMP and short-circuits protein kinase A–mediated ASM relaxation (12). Moreover, GABA_B activation leads to increased intracellular calcium (comparable with that induced by G_q-coupled GPCRs such as the M3 muscarinic acetylcholine receptor) and potentiates ASM contraction stimulated by other contractile agonists (13), thus limiting the utility of GABA_B in the treatment of airflow obstruction in asthma.

GABA_A channels are hetero-oligomeric with multiple possible combinations of distinct subunits, which creates challenges in developing selective agonists. Fortunately, GABA_A on human ASM cells is limited to pentameric oligomers of only $\alpha4$ and $\alpha5$ subunits (among α-subunit variants) (8). Emala and colleagues have previously demonstrated that selective pharmacological targeting of these subunits evokes airway relaxation (9, 14, 15) and also limits the development of airway hyperresponsiveness and inflammation in murine models (14, 16). Finally, *in vivo* studies in murine models of asthma have established that deletion of the GABA_A $\alpha4$ subunit in mice results in exacerbation of airway inflammation, underscoring its relevance to mitigating asthma features beyond airway constriction (17).

In this issue of the *Journal*, Perez-Zoghbi and colleagues (pp. 482–490) report on their studies in which they sought to characterize a recently developed (16) and highly efficacious agonist (PI320) of GABA_A that can also improve tissue specificity, thus avoiding nonspecific activation at central sites (18). PI320 is a modified (polyethylene glycol chain) derivative of an existing GABA_A ligand, the imidazodiazepine MIDD0301. Using a combination of *in vivo* (forced oscillation technique, flexiVent; SCIREQ) and *ex vivo* (murine precision-cut lung slices [PCLS]) approaches, they demonstrate that PI320 can inhibit methacholine-induced airway contraction. Using transgenic mice that express the intracellular Ca^{2+} sensor GCaMP6f, the authors elegantly show that PI320 inhibits contractile agonist–induced Ca^{2+} oscillations. To gain mechanistic insight into PI320-mediated inhibition of agonist-induced Ca^{2+}, they also loaded murine PCLS with membrane-permeable caged inositol 1,4,5-trisphosphate (IP_3) to examine the contribution of IP_3 receptors on the sarcoplasmic reticulum. By combining flash photolysis (using an ultraviolet-light illuminator) to uncage IP_3 in PCLS in tandem with monitoring changes in the airway lumen area with phase-contrast microscopy, the authors demonstrate that PI320 significantly inhibits the airway constriction induced by uncaging IP_3. Also, PI320 does not inhibit caffeine-induced murine PCLS contraction. Collectively, these experiments demonstrated that PI320 regulates Ca^{2+} mobilization mediated through IP_3 receptors (and not ryanodine receptors) on the sarcoplasmic reticulum.

The physiological approaches applied by the authors are essential toward establishing the relevance of GABAergic functional outcomes in ASM. However, as noted by the authors, preliminary studies suggest that PI320-mediated relaxation (at least in murine tissues) appears to be independent of GABA_A. Although this may be partly due to the differential pharmacokinetic and pharmacodynamic behavior of PI320, muscimol (GABA_A agonist), and flumazenil (GABA_A antagonist) in different species, additional studies with human tissues (e.g., PCLS) are warranted to establish the clinical relevance of targeting GABA_A with PI320. These *in vivo* and *ex vivo* approaches could also significantly benefit from supplementation with *in vitro* live-cell micromechanical approaches that allow for the integration of pharmacological and gene-deletion–based approaches to detail various nuances associated with receptor activation and ASM cell function. Further, reductionist approaches aimed at linking the proximal events after GABA_A activation to the downstream signaling driving ASM relaxation will provide much needed clarity into the mechanistic action of PI320. Potentiation of GABA_A channels

associated with the central nervous system is always a concern, because it may lead to undesirable side effects, including sedation or drug dependence. However, it should be noted that $GABA_A$ α subunit variants that contribute toward such outcomes (α1, α2, and α3) (19) may not be activated by α4- and α5-subunit selective agonists. Although the solubility profile of PI320 (owing to polyethylene glycol chain) is an improvement, $GABA_A$ α subunit selectivity is unclear.

In summary, these studies by Perez-Zoghbi and colleagues underscore the importance of continuous refinements in ligand development to improve the receptor selectivity and efficacy profile of novel targets for providing relief from the pathophysiological features of asthma. These approaches could help overcome possible nonspecific interactions and examine relative contributions of other receptors such as the ovarian cancer OGR1 (G protein-coupled receptor-1) (20–23) and the mitochondrial translocator protein (also termed the peripheral benzodiazepine receptor) that can also bind certain benzodiazepine-class drugs (24). Future studies seeking to clarify the relaxation mechanisms for benzodiazepine in ASM cells may help uncover unique signaling networks shared by $GABA_A$, OGR1, and the translocator protein that can be exploited to refine therapeutic strategies for asthma control. ■

Ajay P. Nayak, Ph.D.
Center for Translational Medicine
and
Department of Medicine
Thomas Jefferson University
Philadelphia, Pennsylvania

Steven S. An, Ph.D.
Rutgers Institute for Translational Medicine and Science
New Brunswick, New Jersey
and
Rutgers-Robert Wood Johnson Medical School
The State University of New Jersey
Piscataway, New Jersey

ORCID IDs: 0000-0003-3437-0096 (A.P.N.); 0000-0003-4723-1888 (S.S.A.).

References

1. Axelrod J, Whitby LG, Hertting G. Effect of psychotropic drugs on the uptake of H3-norepinephrine by tissues. *Science* 1961;133:383–384.
2. Hertting G, Axelrod J. Fate of tritiated noradrenaline at the sympathetic nerve-endings. *Nature* 1961;192:172–173.
3. Nelson H, Mandiyan S, Nelson N. Cloning of the human brain GABA transporter. *FEBS Lett* 1990;269:181–184.
4. Chebib M, Johnston GA. The 'ABC' of GABA receptors: a brief review. *Clin Exp Pharmacol Physiol* 1999;26:937–940.
5. Gladkevich A, Korf J, Hakobyan VP, Melkonyan KV. The peripheral GABAergic system as a target in endocrine disorders. *Auton Neurosci* 2006;124:1–8.
6. Mizuta K, Xu D, Pan Y, Comas G, Sonett JR, Zhang Y, *et al.* GABAA receptors are expressed and facilitate relaxation in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L1206–L1216.
7. Osawa Y, Xu D, Sternberg D, Sonett JR, D'Armiento J, Panettieri RA, *et al.* Functional expression of the GABAB receptor in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2006; 291:L923–L931.
8. Gallos G, Yim P, Chang S, Zhang Y, Xu D, Cook JM, *et al.* Targeting the restricted α-subunit repertoire of airway smooth muscle GABAA receptors augments airway smooth muscle relaxation. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L248–L256.
9. Gallos G, Yocum GT, Siviski ME, Yim PD, Fu XW, Poe MM, *et al.* Selective targeting of the α5-subunit of GABAA receptors relaxes airway smooth muscle and inhibits cellular calcium handling. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L931–L942.
10. Gallos G, Gleason NR, Zhang Y, Pak SW, Sonett JR, Yang J, *et al.* Activation of endogenous GABAA channels on airway smooth muscle potentiates isoproterenol-mediated relaxation. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L1040–L1047.
11. Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, *et al.* Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K+ channels. *Proc Natl Acad Sci USA* 1998;95:14991–14996.
12. Billington CK, Penn RB. Signaling and regulation of G protein-coupled receptors in airway smooth muscle. *Respir Res* 2003;4:2.
13. Mizuta K, Mizuta F, Xu D, Masaki E, Panettieri RA Jr, Emala CW. Gi-coupled γ-aminobutyric acid-B receptors cross-regulate phospholipase C and calcium in airway smooth muscle. *Am J Respir Cell Mol Biol* 2011;45:1232–1238.
14. Forkuo GS, Guthrie ML, Yuan NY, Nieman AN, Kodali R, Jahan R, *et al.* Development of GABAA receptor subtype-selective imidazobenzodiazepines as novel asthma treatments. *Mol Pharm* 2016;13:2026–2038.
15. Yocum GT, Gallos G, Zhang Y, Jahan R, Stephen MR, Varagic Z, *et al.* Targeting the gamma-aminobutyric acid a receptor alpha4 subunit in airway smooth muscle to alleviate bronchoconstriction. *Am J Respir Cell Mol Biol* 2016;54:546–553.
16. Zahn NM, Roni MSR, Yocum GT, Meyer MJ, Webb DA, Mian MY, *et al.* Development of inhaled gabaa receptor modulators to improve airway function in bronchoconstrictive disorders. *ACS Pharmacol Transl Sci* 2022;5:80–88.
17. Yocum GT, Turner DL, Danielsson J, Barajas MB, Zhang Y, Xu D, *et al.* GABAA receptor α_4-subunit knockout enhances lung inflammation and airway reactivity in a murine asthma model. *Am J Physiol Lung Cell Mol Physiol* 2017;313:L406–L415.
18. Perez-Zoghbi JF, Sajorda DR, Webb DA, Arnold LA, Emala CW, Yocum GT. Imidabenzodiazepine PI320 relaxes mouse peripheral airways by inhibiting calcium mobilization. *Am J Respir Cell Mol Biol* 2022;67:482–490.
19. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci* 2008;9:331–343.
20. Huang XP, Karpiak J, Kroeze WK, Zhu H, Chen X, Moy SS, *et al.* Allosteric ligands for the pharmacologically dark receptors GPR68 and GPR65. *Nature* 2015;527:477–483.
21. Nayak AP, Deshpande DA, Shah SD, Villalba DR, Yi R, Wang N, *et al.* OGR1-dependent regulation of the allergen-induced asthma phenotype. *Am J Physiol Lung Cell Mol Physiol* 2021;321: L1044–L1054.
22. Nayak AP, Pera T, Deshpande DA, Michael JV, Liberato JR, Pan S, *et al.* Regulation of ovarian cancer G protein-coupled receptor-1 expression and signaling. *Am J Physiol Lung Cell Mol Physiol* 2019; 316:L894–L902.
23. Pera T, Deshpande DA, Ippolito M, Wang B, Gavrila A, Michael JV, *et al.* Biased signaling of the proton-sensing receptor OGR1 by benzodiazepines. *FASEB J* 2018;32:862–874.
24. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, *et al.* Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* 2006;27: 402–409.