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Anxiolytics for Bronchodilation: Refinements to GABA

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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 α4 and α5 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 $GABA_A$ α4 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

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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. ■

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