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Diversity of neuronal inhibition— a path to novel treatments for neuropsychiatric disorders

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Benzodiazepines have been used for more than half a century for their anxiolytic, hypnotic, anticonvulsant and myorelaxant properties. Benzodiazepines act as positive allosteric modulators of the γ -aminobutyric acid (GABA) type-A receptor (GABA_AR), amplifying inhibitory neurotransmission. While the use of benzodiazepines provides rapid and effective symptom relief, their use as therapeutic agents has declined over the years due to their unfavorable side-effect profile, e.g. sedation when used for daytime anxiolysis, falls related to muscle relaxation and ataxia, development of tolerance, and sometimes dependence after long-term use. While clinically used benzodiazepines modulate several GABA_AR subtypes non-selectively, research over the last 25 years has helped to identify and functionally characterize individual GABA_AR subunits and subtypes. In this Viewpoint, we provide a synopsis¹ of the research on the role of GABA_ARs in neuropsychiatric disorders and their treatment, with consideration of targeting individual GABA_AR subtypes to achieve novel pharmacological profiles.

GABA_AR structure and function

GABA_ARs are the major inhibitory neurotransmitter receptors in the brain. They are pentameric complexes with GABA and - in most cases -benzodiazepine binding sites (see Fig.). GABA_AR subunits are divided into eight subunit classes based on sequence homology (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π , and ρ 1-3)¹. *In vitro* and *in vivo* studies have determined that different subunits confer distinct receptor localization, physiological function, and pharmacology. Benzodiazepines act as positive allosteric modulators at GABA_ARs containing α 1, α 2, α 3 or α 5 subunits (abbreviated α 1-GABA_ARs, etc.)². α 4-GABA_ARs and α 6-GABA_ARs do not bind classical benzodiazepines. A single residue in the N-terminal extracellular segment of the α subunit determines sensitivity to classical benzodiazepines: α subunits with a histidine (α 1, α 2, α 3, α 5) are sensitive to diazepam, while α subunits with an arginine (α 4, α 6) are insensitive to diazepam. The generation of gene targeted knock-in

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In the last three years, Dr. Rudolph has provided professional services for Sunovion Pharmaceuticals and Concert Pharmaceuticals.

¹We have chosen to cite review articles in order to cover a broader range of material. Please see these review articles for the citations of original works.

mice with histidine-to-arginine point mutations, selectively rendering mutant α subtypes insensitive to benzodiazepines while maintaining GABA sensitivity ($\alpha 1H101R$, $\alpha 2H101R$, $\alpha 3H126R$ and $\alpha 5H101R$ mice), and the development of compounds with some subtype-selectivity have helped elucidate which specific α subunits are responsible for the behavioral effects of benzodiazepines². Furthermore, the generation of mice carrying knockout alleles of different GABA_AR subunits has contributed to our understanding of the roles of different GABA_AR subunits in behavior and disease.

GABA_ARs and Anxiety

Studies employing GABA_AR knock-in mice revealed that the sedative and amnesic actions of diazepam are abolished in $\alpha 1H101R$ mice, while the anxiolytic-like action remains intact². In contrast, in $\alpha 2H101R$ mice the anxiolytic-like action of diazepam is absent, while the sedative action is not affected². This indicates that $\alpha 1$ -GABA_ARs mediate sedation, and $\alpha 2$ -GABA_ARs mediate anxiolysis. The role of $\alpha 2$ -GABA_ARs in anxiety is also demonstrated in $\alpha 2$ -global knockout mice which exhibit anxiety-like phenotypes (see³ for a review). In humans, ligand-binding studies revealed GABA_AR deficits in the brain in different anxiety disorders⁴. However, human genetic studies do not provide clear evidence for a contribution of *GABRA2* (the gene encoding the $\alpha 2$ subunit) polymorphisms to anxiety³.

GABA_AR subtype-selective compounds that are $\alpha 1$ -sparing have shown some promise in animal models of anxiety. The $\alpha 2/\alpha 3/\alpha 5$ partial allosteric modulator, L-838,417, was anxiolytic but not sedating in rodents and primates. Furthermore, the $\alpha 2/\alpha 3$ partial positive allosteric modulator, MK-0777, was anxiolytic but not sedating in Phase 2 studies in humans. However, development was halted due to the appearance of cataracts in animals². Despite this, the findings reviewed here support the development of $\alpha 2$ -selective, $\alpha 1$ -sparing positive modulators as non-sedating anxiolytics.

GABA_AR and Depression

Behavioral studies employing gene-targeted mice have begun to elucidate the role of specific GABA_ARs in depression. In particular, heterozygous knockout of the $\gamma 2$ subunit (which is contained in 90% of GABA_ARs), and homozygous knockout of the $\alpha 2$ subunit result in depressive-like phenotypes in tests of behavioral despair^{3,4}. Alterations in GABA_AR subunit expression are also found in the cortex of mice exposed to stress (a factor in the development of depression) as well as in depressed patients following suicide⁴. However, the genetics of depression is still largely unknown, and there is no evidence so far for strong associations between specific polymorphisms in the GABA system and depression.

As outlined above, behavioral mouse studies indicate a protective physiological role for $\alpha 2$ -GABA_ARs in counteracting depression. Interestingly, when given in combination with fluoxetine, the GABA_AR allosteric modulator, eszopiclone, has a synergistic effect on depression and anxiety symptoms in patients suffering from depression and insomnia⁴. Moreover, there is clinical evidence that the benzodiazepine alprazolam can rapidly relieve

depressive symptoms⁵, suggesting that it may be possible to develop $\alpha 2$ -selective GABAergic drugs as fast-acting antidepressants.

GABA_AR and Schizophrenia

Different GABA_AR subtypes have been implicated in symptoms of schizophrenia. Mice lacking the $\alpha 3$ subunit, or with reduced $\alpha 5$ subunit expression, display deficits in prepulse inhibition (PPI) to acoustic startle, i.e., trivial sensory inputs are not filtered out at the expense of relevant stimuli^{2,6}. While not specific, such deficits are common in schizophrenia. Mice with reduced $\alpha 5$ expression also have a latent inhibition deficit, which may model the failure to show selectivity in learning in patients with schizophrenia². Postmortem studies found that $\alpha 2$ -GABA_ARs are upregulated in a defined subcellular location in the dorsolateral prefrontal cortex of schizophrenic patients, while GABA transporter 1 (GAT1) expression was decreased. Such changes may be interpreted as insufficient compensation in response to a reduced activity of GABAergic interneurons. Insufficient GABAergic modulation of $\alpha 2$ -GABA_ARs at the synapses at the axon initial segment of pyramidal neurons may contribute to working memory dysfunction, a clinical feature of schizophrenia⁷.

These findings suggest that not only $\alpha 3$ - and $\alpha 5$ -GABA_ARs, but also $\alpha 2$ -GABA_ARs may be useful drug targets for different symptoms of schizophrenia. In a small clinical trial, the $\alpha 2/3$ partial positive allosteric modulator MK-0777 was found to improve behavioral and electrophysiological measures of prefrontal function in schizophrenia. However, a larger study reported no cognitive improvement in response to MK-0777². As the efficacy of MK-0777 at $\alpha 2$ -GABA_ARs is only 11% compared to chlordiazepoxide, it is conceivable that an $\alpha 2$ -selective compound with higher efficacy would have more desired effects. Moreover, a small open label study showed that the non-selective partial allosteric agonist bretazenil was efficacious in 40% of patients with acute episodes of schizophrenia, further suggesting that GABA_ARs are potential drug targets for schizophrenia².

GABA_AR and Autism

A general reduction in the density of GABA_ARs has been observed in postmortem brains of autistic patients. More specifically, reduced expression of $\alpha 4$, $\alpha 5$, and $\beta 1$ subunits have been detected⁸. The high incidence of anxiety symptoms and seizures in patients with autism are also suggestive of GABAergic hypofunction. Several genetic mouse models of autism have been found to have deficits in the functions of GABAergic neurons, including heterozygous *Scn1a* (Na_v1.1 channel) knockout mice, which is a model of Dravet's syndrome, a childhood disorder including recurrent intractable seizures, cognitive deficits and autism-spectrum behaviors. These mice exhibit hyperactivity, stereotyped behaviors, social interaction deficits, and impaired context-dependent spatial memory. Interestingly, a very low (subanxiolytic) dose of clonazepam rescued the social interaction and the fear-associated memory deficits in these mice⁹. The GABA_AR subtype(s) underlying these responses are not currently known, nor is it known whether benzodiazepines have similar effects in other genetic mouse models of autism or in patients. Nevertheless, the findings raise the question whether subtype-selective GABAergic compounds can be exploited in the

future to treat core symptoms of autism such as cognitive rigidity and social interaction deficits.

GABA_AR and Addiction

Although benzodiazepines are considered to be safe for short-term use, long-term use is frequently discouraged due to the risk of developing tolerance and physical dependence. In fact, benzodiazepines are among the most commonly abused prescription drugs. The addictive properties of benzodiazepines have been linked to α 1-GABA_ARs, as benzodiazepine-evoked plasticity in dopaminergic neurons of the ventral tegmental area (which is a hallmark of drugs of abuse) is abolished in α 1H101R mice¹⁰. Moreover, α 1-GABA_ARs are required for midazolam preference in oral self-administration studies². Consistent with these findings, in *ex vivo* experiments, the α 1-selective allosteric modulator zolpidem induced plasticity, whereas the α 2/ α 3/ α 5-selective allosteric modulator L-838,417 did not^{2,10}. Thus, α 1-sparing compounds might have a reduced addiction liability.

While the addictive properties of benzodiazepines appear to be critically dependent on α 1-GABA_ARs, α 2-GABA_ARs and α 3-GABA_AR are necessary for the reward-related properties of benzodiazepines. The reward-enhancing action of diazepam on self-stimulation of the medial forebrain bundle is absent in α 2H101R and α 3H126R mice, and reduced in α 1H101R mice³. Polymorphisms in the *GABRA2* gene have been linked to alcohol dependence and to the abuse of illicit substances such as cocaine and heroin³, but the mechanism behind this association is unknown. A better knowledge of the roles of individual GABA_AR subtypes in reward-and addictive-related behaviors will provide insights for the development of novel GABAergic compounds with less abuse liability, as well as novel therapeutics to treat addiction and drug abuse.

Conclusions

The last two decades have seen remarkable progress in elucidating the diversity of neuronal inhibition as exemplified by GABA_AR subtypes. There is extensive evidence for deficits in GABAergic inhibition in a variety of neuropsychiatric disorders. The more targeted reversal of such deficits may be possible with GABA_AR subtype-selective compounds, which may have fewer side effects or properties that are not apparent in classical benzodiazepines.

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Figure. GABA_ARs: composition, expression, and contribution to neuropsychiatric disorders

(A) GABA_ARs are defined by the assembly of five subunits, creating a pore through which Cl⁻ ions flow down their concentration gradient to mediate fast synaptic inhibition. The most common subunit assembly is depicted here with 2α, 2β, and 1γ subunit. The receptor is also defined by the presence of GABA and benzodiazepine binding sites, which are formed by α and β or α and γ subunits respectively. Upper panel reprinted by permission from Macmillan Publishers, Ltd: Nature Reviews Neuroscience, 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. (B) The four α subunits which bind classical benzodiazepines (α1, α2, α3, and α5) have distinct expression patterns within the brain. Image reproduced by permission of ASPET from Mohler H, Fritschy JM, Rudolph U. A new benzodiazepine pharmacology. *J Pharm and Exp Ther.* 2002;300:2-8. (C) A simplified summary table indicating potential roles of α subunits in neuropsychiatric disorders or their treatment. The table highlights α subunits based on converging evidence from genetically modified mice and selective compounds. However, many cells in the table have not yet been explored completely. BZ = benzodiazepine.