1521-0103/391/3/387–388$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.124.002299
J Pharmacol Exp Ther 391:387–388, December 2024

Viewpoint

Precisely Providing Analgesia through Selectively Targeting the $GABA_A$ α2/α3 Subtypes

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter within the brain regulating neuronal excitability through a family of GABA receptors including $GABA_A$. Despite preclinical evidence, $GABA_A$ receptor agonists are antinociceptive (Kendall et al., 1982; Malan et al., 2002) the $GABA_A$ receptor is largely untargeted to develop analgesics due to the concomitant sedative, anesthetic, and amnestic effects occurring with orthosteric drugs, such as midazolam, that bind to the $GABA_A$ receptor active site.

Structurally, the $GABA_A$ receptor is composed of six different subtypes spanning α1 through α6. The α2 and α3 subtypes produce sedation, and in particular, the α2 subtype of the $GABA_A$ receptor also contributes toward anxiolysis. This is opposed to α subtypes of the $GABA_A$ receptor that when activated contribute toward anesthesia or amnesia (Fig. 1). Therefore, allosteric modulation of the $GABA_A$ receptor presents an opportunity to develop small molecules to specifically target $GABA_A$ subtypes that lead to analgesic effects while potentially leaving other $GABA_A$ receptor subtypes unopposed.

In this regard, Lewter and colleagues within this issue of *JPET* describe how targeting the α2/α3 subunit of the $GABA_A$ receptor with selective positive allosteric modulators (PAMs) may be a solution to targeting the $GABA_A$ receptor to provide analgesia without amnesia or anesthesia (Lewter et al., 2024). The authors find that the $GABA_A$ PAMs, KRM-II-81, and NS16085 provide antinociceptive qualities in a dose- and time-dependent manner when male Sprague–Dawley rats are subjected to complete Freund's adjuvant or chronic nerve constriction models. The behavioral studies conducted also highlighted the differences between the α2/α3 PAMs and midazolam, illustrating that the α2/α3 PAMs for the $GABA_A$ receptor, unlike midazolam, produced analgesia without unwanted side effects such as amnesia. Further, the $GABA_A$ antagonist flumazenil prevented the antinociceptive effects previously detected with KRM-II-81, NS-16085, and midazolam, demonstrating the antinociceptive effects of $GABA_A$ receptor PAMs are specific to the $GABA_A$ receptor. Taken together, this data identifies that KRM-II-81 and NS-16085 have the potential for modulating nociception via the $GABA_A$ α2/α3 subtypes without amnesia or anesthesia, opening the door to developing drugs that can precisely modify the $GABA_A$ receptor to treat pain.

Developing $GABA_A$ α2/α3 PAMs to treat pain shows tremendous promise. The extensive knowledge of how the $GABA_A$ receptor impacts behavior may provide an advantage when developing selective modulators for $GABA_A$ subtypes as compared to research focused on developing nonopioid pain therapeutics for other targets (Giancotti et al., 2024; Sato et al., 2024; Stuertz et al., 2023; Zambelli and Gross, 2023; Angelia et al., 2023). However, as the study by Lewter only used male rodents, the importance of identifying potential differences in female and in aged rodent models cannot be underscored, as there are notable differences in $GABA_A$ subunit expression, including α2/α3 that are sex- and age-dependent (Pandya et al., 2019). Also, KRM-II-81 and NS16085 had no impact on thermal hyperalgesia, which is important to understand further as newly developed drugs such as the α2/α3 $GABA_A$ PAMs should ideally provide a similar analgesic profile as opioids to replace them.

As translating α2/α3 $GABA_A$ receptor PAMs for use in humans is the ultimate goal, several other factors must be considered when developing these and other drugs to treat chronic pain. As multimodal approaches to analgesia are frequently used, understanding how treatment with α2/α3 PAMs may impact dosing with other analgesics that have sedative properties, such as gabapentin, should be considered. Further, the $GABA_A$ receptor is the primary target that induces the hypnotic state for intravenous anesthetics

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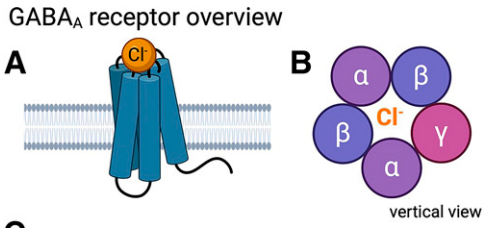
This work is supported by funding from the Foundation for Anesthesia Education and Research (to C.L.G.) and National Institutes of Health National Institute of General Medical Sciences [Grant GM119522] (to E.R.G.).
E.R.G. is a consultant for Chiima Therapeutics and an associate editor for *JPET*.
dx.doi.org/10.1124/jpet.124.002299.

ABBREVIATIONS: GABA, gamma-aminobutyric acid; PAM, positive allosteric modulator.



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Receptor subunit	Effects observed
α1	anesthesia and anticonvulsivant
α2 and α3	anxiolytic
α4*	anxyolitic/sedative
α5	memory impairment
α6*	anxyolitic/sedative

Fig. 1. The GABA$_A$ receptor and subtypes. (A) The GABA$_A$ receptor is a ligand-mediated chloride channel. The GABA$_A$ receptor subtypes form pentameric combinations composed of 19 different subunits, including α, β, γ, δ, ε, π, θ, and ρ. B. The majority of GABA$_A$ receptors consist of 2 α, 2 β, and 1 γ subunit. C. In particular, the α1-α3 and α5 subtypes respond to classical benzodiazepines such as midazolam as opposed to the α4 and α6 subunits, which are insensitive (*). The α5 subunit is mainly expressed within the hippocampus, contributing to memory impairment after general anesthesia, and the α6 subunit is mainly expressed within the cerebellum.

(Forman and Miller, 2016), volatile anesthetics (Woll et al., 2018), and alcohol (Lobo and Harris, 2008). As such, the use of GABA$_A$ PAMs to treat chronic pain should examine at a minimum whether there are additive sedative effects when combined with alcohol. When using α2/α3 GABA$_A$ receptor PAMs to treat chronic pain, determining whether tolerance may occur with long-term treatment is also needed. Developing tolerance to analgesia would require escalated doses of α2/α3 GABA$_A$ receptor PAMs, which may lead to unwanted effects such as amnesia or anesthesia. The addictive properties of PAMs should also be considered with preclinical assessment by conditioned place preference or self-administration. Tests should also be considered to assess whether physical dependence occurs with long-term use and whether abruptly stopping PAMs after chronic use could lead to withdrawal. Even within the context of these additional questions that will need to be addressed prior to translation, the findings of Lewter et al. are an exciting idea that can lead the way to developing drugs targeting specific subtypes of the GABA$_A$ receptor to treat pain.

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References

Angelia J, Kandasamy R, Weng X, and Pecic S (2023) Dual sEH/FAAH inhibitors as a promising therapeutic strategy for the treatment of chronic pain. *J Pharmacol Exp Ther* **385:**90.
Forman SA and Miller KW (2016) Mapping general anesthetic sites in hetromeric g-aminobutyric acid type a receptors reveals a potential for targeting receptor subtypes. *Anesth Analg* **123:**1263–1273.
Giancotti LA, Lauro F, Olayide I, Zhang J, Arnatt CK, and Salvemini D (2024) 12-(S)-Hydroxyeicosatetraenoic acid and GPR31 signaling in spinal cord in neuropathic pain. *J Pharmacol Exp Ther* **388:**765–773.
Kendall DA, Browner M, and Enna SJ (1982) Comparison of the anti-nociceptive effect of gamma-aminobutyric acid (GABA) agonists: evidence for a cholinergic involvement. *J Pharmacol Exp Ther* **220:**482–487.
Lewter LA, Woodhouse K, Tiruveedhula PB, Cook JM, and Li JX (2024) Antinociceptive effects of a2/a3-subtype-selective GABAA receptor positive allosteric modulators KRM-II-81 and NS16085 in male rats: behavioral specificity. *J Pharmacol Exp Ther* **391**:389–398 DOI: 10.1124/jpet.123.002070.
Lobo IA and Harris RA (2008) GABA(A) receptors and alcohol. *Pharmacol Biochem Behav* **90:**90–94.
Malan TP, Mata HP, and Porreca F (2002) Spinal GABA(A) and GABA(B) receptor pharmacology in a rat model of neuropathic pain. *Anesthesiology* **96:**1161–1167.
Pandya M, Palpagama TH, Turner C, Waldvogel HJ, Faull RL, and Kwakowsky A (2019) Sex- and age-related changes in GABA signaling components in the human cortex. *Biol Sex Differ* **10:**5.
Sato A, Yasukochi S, Iwanaka N, Yamauchi T, Tsuruta A, Koyanagi S, and Ohdo S (2024) Dosing time-dependent difference in the suppressive effect of empagliflozin on the development of mechanical pain hypersensitivity in diabetic mice. *J Pharmacol Exp Ther* **390**:177–185 DOI: 10.1124/jpet.123.001856.
Stuertz ED, Olayide I, Braden K, Salvemini D, and Arnat CK (2023) Development of GPR183 antagonists to treat neuropathic pain. *Journal of Pharmacology and Experimental Therapeutics* 385(Suppl 3):364.
Woll KA, Zhou X, Bhanu NV, Garcia BA, Covarrubias M, Miller KW, and Eckenhoff RG (2018) Identification of binding sites contributing to volatile anesthetic effects on GABA type A receptors. *FASEB J* **32:**4172–4189.
Zambelli VO and Gross ER (2023) Fixing a broken heart opens the door to developing KATP channel agonists as pain relievers. *J Pharmacol Exp Ther* **387:**15–17.