GABA$_B$ Thera$_P$

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Abstract

In the mammalian central nervous system, gamma-aminobutyric acid (GABA) is the primary inhibitory signaling molecule. One receptor for this molecule, GABAB, has been linked to feelings of calmness, as well as mental disorders such as alcoholism and depression. Pharmaceutical compounds that bind the GABAB receptor are currently used to treat muscle spasticity and various types of addiction. However, excessive activation of this receptor can hinder muscle function. Activation of the metabotropic GABAB receptor by GABA influences neuronal activity by coupling with G proteins to activate a signaling cascade that leads to downstream effects including the modulation of various ion channels. The GABAB receptor is a dimer composed of two different subunits (GBR1 and GBR2), each with 7 helices within the membrane and an extracellular domain that binds GABA. Only the GBR1 subunit directly binds the GABA molecule and other ligands with a similar structure. However, recent studies have shown that GBR2 can affect the efficiency of GABA binding to GBR1. In addition, the GBR2 subunit activates the G protein after GABA binds, leading to various downstream effects. One well known effect is the opening of potassium channels, hyperpolarizing the cell, preventing action potentials from firing, and ultimately stopping neurotransmitter release. Using 3D printing technology, the Kettle Moraine SMART (Students Modeling A Research Topic) Team has modeled the GABAB receptor to study its structure to determine therapeutic possibilities. GABAB receptors' widespread importance in the nervous system may lead to new uses in the neurological and medical fields.

Background Information

**Neurotransmitter:** a substance in the body that carries a signal from one nerve cell to another

Synapse: the space between two nerve cells where neurotransmitters are released and received

Process: the presynaptic nerve cell releases neurotransmitters into the synapse, and they are accepted by the dendrites of adjacent cells. The acceptance of these neurotransmitters triggers a response within the cell that leads to the production of an action potential down the axon, leading to the release of the next cell's neurotransmitters.

Application to Medicine

The activation of GABA$_B$ influences the reward process within the human mind, making it effective in modulating addictions. Multiple clinical investigations have been conducted in which subjects addicted to alcohol, cocaine, methamphetamines, GHB, nicotine, and opiates were administered doses of GABA$_B$ agonists; including baclofen, vigabatrin, and tiagabine. Conclusive results were drawn in cases involving alcohol, nicotine, and opiates; all of them suggesting that agonists of GABA$_B$ receptors seem to have potential as treatments in the process of withdrawal. After agonists were dispensed, subjects were found to have increased tolerance and less relapses. Modulators of GABA$_B$ receptors may someday serve as treatments to addiction and various narcotic dependencies.

G Protein Coupled Receptors
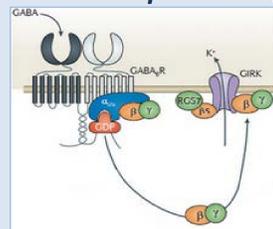
G protein coupled receptors are transmembrane proteins that bind signaling molecules in their extracellular regions. Once a ligand has been bound, the receptor changes shape, triggering the interaction with the G protein. G proteins are heterotrimeric -- three subunits; alpha, beta, and gamma -- and bind to guanosine diphosphate (inactive) and guanosine triphosphate (active). Once the G protein has been activated, its subunits are able to split off and relay several secondary signals to other proteins.

Modified image from "Regulation of neuronal GABA$_B$ receptor functions by subunit composition"

Structure

- 2 subunits in this heterodimeric receptor, GBR1 and GBR2
- GBR1 and GBR2 both have 7 transmembrane helices, connected by their carboxy termini
- GBR1 receives GABA, and GBR2 modulates its binding

Model Description

The sidechains pointed out are Tyr 118 and Asp 256. Tyr 118 is a mutation that decreases the density of GIRK channels that GABA$_B$ controls. On the 4F11 and 4F12 protein data bank files amino acid 256 is aspartic acid. However, when mutated into asparagine, it also decreases the density of GIRK channels. These two amino acids show that mutations and changes to GBR2 directly influences how GABA interacts with the pathway (despite binding exclusively to GBR1), as explained by the data.


4f12.pdb
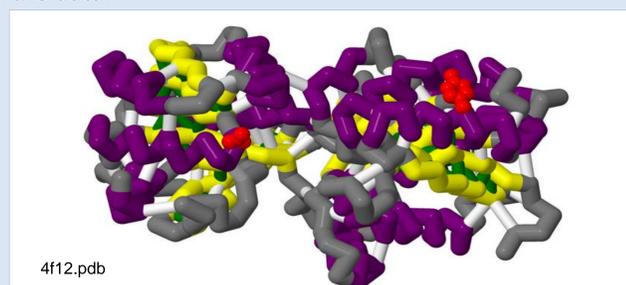
The Importance of the GBR2 Subunit

Experiment: Doses of unmarked GABA were introduced to a system of tritium-tagged GABA ([^{3}H]-GABA) bound to the GABA$_B$ receptor. The amount of [^{3}H]-GABA still bonded to the receptor was then measured, and the percentage relative to the total amount of agonist binding was graphed.. This was done in the absence of the GBR2 subunit (black) and again in its presence (red).


Results: With the GBR2 subunit, the introduced GABA is more able to inhibit the binding of the [^{3}H]-GABA. Since the graph is on a logarithmic scale, this can be seen through the IC-50. The red curve is to the left of the black curve, demonstrating the lower required concentration in the presence of the GBR2 subunit.

Implications: The GABA$_B$ receptor is heterodimeric, but ligands only bind to the GBR1 subunit. This experiment shows that the GBR2 subunit functions as a modulator that increases the strength and efficiency of the binding site.

Modified image from "Structure and functional interaction of the extracellular domain of human GABAB receptor GBR2"
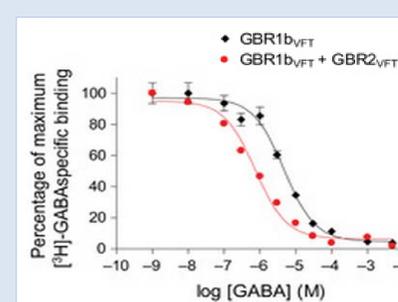
The Effect of Mutations
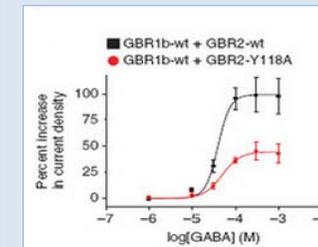
Experiment: Unmarked GABA was introduced to a system where tritium-tagged GABA ([^{3}H]-GABA)was bound to the GABA$_B$ receptor in varying concentrations, and the percentage of [^{3}H]-GABA that remained bound to the receptor was graphed relative to the introduced concentration. This was done with only the GBR1 subunit (black) and with the both subunits and a mutation of the Tyr118 in GBR2 to alanine (red).

Results: While it has previously been established that, unmutated, GBR2 increases the binding potential of the GBR1 subunit, the mutation of Tyr118 into alanine nullifies that increased potential, as seen by the nearly identical graphs. With the mutation, the ability of the entire receptor to bind GABA is just as efficient as the GBR1 subunit by itself, essentially rendering the GBR2 subunit useless in that regard.

Implications: Tyr118 is crucial to the function of GBR2 in amplifying the binding potential of the GBR1 subunit, as without it the GBR2 subunit does not affect binding whatsoever.

Experiment: Systems involving GABA$_B$ and GIRK channels are compared when having no GABA, the wild type of both GBR1 and GBR2, and the wild type of GBR1 with the Asn 256 mutation on GBR2. The top graph depicts both subunits' wild type and the bottom graph shows the Asn 256 mutation. These graphs show the I-V relationship and current density in the absence (black) and presence (green) of GABA.

Results: Similar to the data concerning Tyr 118, the binding density is lower with Asn 256 than the wild-type GBR2 and higher than the control, meaning that it is less efficient with this mutation to the R2 subunit.

Implications: This is further proof that GBR2 is vital to the function of the GABA$_B$ receptor, despite not binding directly with GABA. Although it does not influence the binding strength of GBR1, this mutation weakens the downstream effects that the binding of GABA can have.
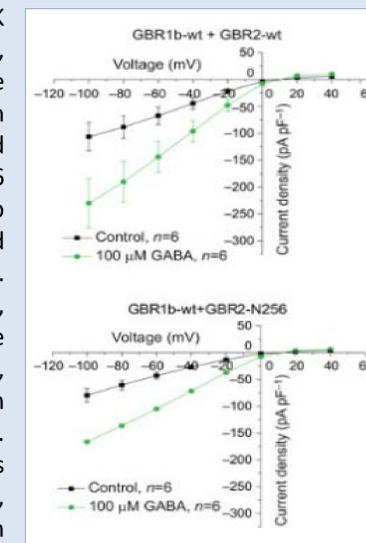
Modified images from "Structure and functional interaction of the extracellular domain of human GABAB receptor GBR2"

Conclusion

GABA$_B$ and its two receptor subunits, GBR1 and GBR2, have a versatile role in modulating many downstream neural channels and processes. Despite a lack of direct binding to ligands, the GBR2 subunit is instrumental in modulating GBR1's ligand interactions. The GABA$_B$ receptor molecule has also been found to have a connection to addiction; a comprehensive understanding of this protein is vital to the growth of medical treatments for many forms of addiction.

References
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