GIRK2 and $GABA_BR1$ Downregulate in Response to TTX as GIRK2, $GABA_BR1$, and $GABA_BR2$ Are Not Affected by BC Treatment

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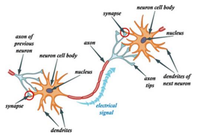
PRECS Phenotypic Plasticity Research Experience for Community College Students

1. Introduction

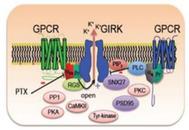
- This project focuses on the **homeostatic plasticity** of neurons in the brain.
- Homeostatic plasticity is the response neurons undergo to regulate changes in excitability levels and bring the cells back to homeostasis.
  - The neurons will either increase or decrease their rate of firing to offset changes in excitability levels that occur. (Lüscher, Christian and Paul A. Slesinger, 2010)
- Without this mechanism, many neurological disorders occur due to irregular firing of neurons, such as epilepsy, Alzheimer's, schizophrenia, etc.
  - Through a better understanding of homeostatic plasticity on the molecular level, improved treatments or a cure can be developed for these diseases.



Neurons communicate by transmitting electrical energy across the synapse (space between 2 neurons) from the axon of the first neuron to the dendrite of the next neuron. Study.com

Here are two major proteins that are responsible for preventing neurons from firing:

- GIRK (G protein-gated, inwardly rectifying potassium) channels are contained at the neuron's synapse.
  - These inhibit action potentials by limiting synaptic transmission.
  - When the channels open, potassium ions flow out, hyperpolarizing the cell (making the overall charge more negative). This prevents the neuron from reaching the threshold value it needs to fire.
- $GABA_B$ (gamma-aminobutyric acid) receptors are major inhibitory neurotransmitters.
  - These inhibit action potentials by chemically inducing GIRK channels to stay open. (Chung, Hee Jung, et al, 2008)



*GABA is a neurotransmitter that will bind to $GABA_B$ receptors, which are G protein-coupled receptors (GPCR). Part of the G-protein inside the receptor will dissociate and bind to the GIRK channel, causing it to stay open. (*Lüscher, Christian and Paul A. Slesinger, 2010)

To study how GIRK channels and $GABA_B$ receptors respond to changes in neuronal excitability levels, two different neurotoxins that cause the neurons to either fire rapidly or slow down were added to the cells.

- Tetrodotoxin (TTX)
  - It inhibits neuronal firing by **blocking** sodium channels.
    - Sodium channels depolarize the cell (make it more positive), letting it reach the electrical potential the cell needs to fire.
- Bicuculline (BC)
  - This allows for more synaptic transmission by blocking $GABA_B$ receptors from binding to GIRK channels that would normally inhibit action potentials.

2. Main Question

How will GIRK channels and $GABA_B$ receptors respond when neurons become excited or inhibited?

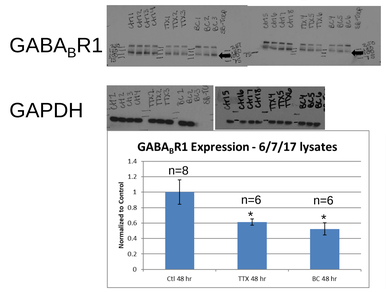
- For each case, will there be more or fewer of these proteins present in the cell to offset the changes in electrical activity?

3. Methods

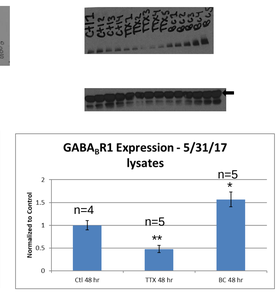
- Treated hippocampal cultured neurons from rat brains with deionized water (control) and neurotoxins: TTX (inhibitory) and bicuculline (excitatory)
- Performed Western Blotting for GIRK2, $GABA_BR1$, $GABA_BR2$, and GAPDH proteins
  - GAPDH used as comparison tool
- Used ImageJ software to quantify results

4. Results

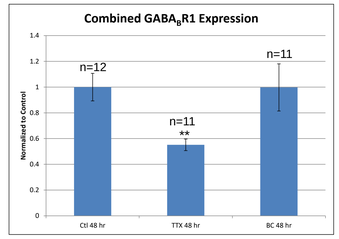
Prolonged blockade of neuronal activity by TTX decreases $GABA_BR1$ expression in hippocampal neurons.



The amount of $GABA_BR1$ significantly decreased for both TTX and BC treatments.

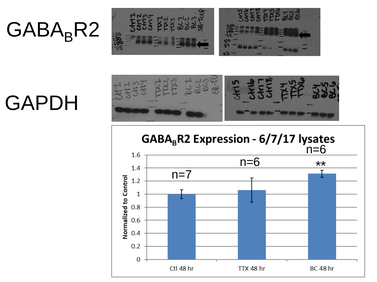


$GABA_BR1$ expression decreased with TTX treatment and increased with BC treatment.

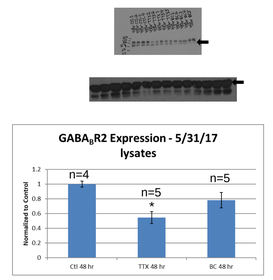


Overall, only TTX significantly reduced $GABA_BR1$ expression, while there was no significant change with BC treatment.

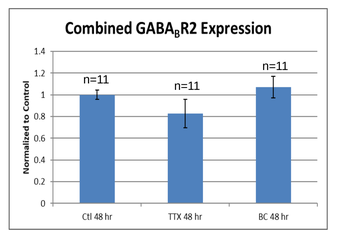
Neither prolonged blockade by TTX nor excitation by BC of neuronal activity affects $GABA_BR2$ expression in hippocampal neurons.



Here BC treatment increased $GABA_BR2$ expression.

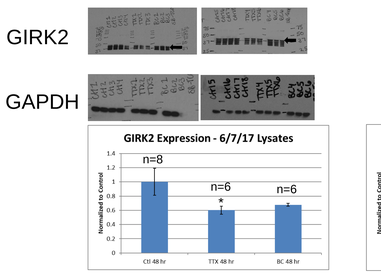


This TTX treatment decreased the amount of $GABA_BR2$ present.

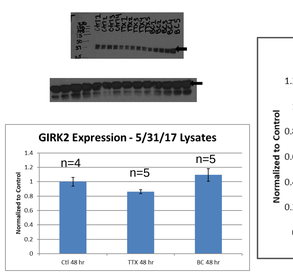


Overall, there was not a significant change of $GABA_BR2$ expression for either treatment.

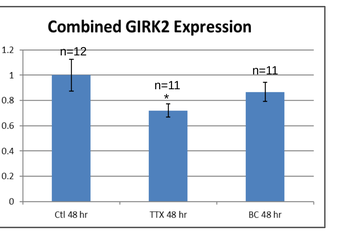
Prolonged blockade of neuronal activity by TTX decreases GIRK2 expression in hippocampal neurons.



GIRK2 expression decreased with TTX treatment.



There was no significant change for either treatment.



Overall, TTX treatment significantly reduced the amount of GIRK2 expression.

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5. Conclusion

Prolonged activity blockade of 48 hour TTX treatment significantly reduced $GABA_BR1$ and GIRK2 expression. This supports the idea that because these two proteins inhibit action potentials, there will be fewer of them found in the cell to offset the inhibition caused by TTX. However, there was no change in expression for $GABA_BR2$. In order to function, $GABA_BR2$ and $GABA_BR1$ rely on one another. Perhaps the decrease in $GABA_BR1$ expression is enough to offset the inhibition by TTX.

Prolonged activity excitation of 48 hour BC treatment resulted in no significant change for $GABA_BR1$, $GABA_BR2$, and GIRK2 expressions. Although their expressions may not have changed, it is possible that their activity could still be increased.

6. Future Direction

Due to a lack of usable primary antibodies for GIRK1, this protein was not studied in this project. Because it is one of the more present subunits of GIRK channels in the brain along with GIRK2, GIRK1 would be a strong protein of interest for a future project.

Previous studies have shown that GIRK2 expression depends on serine-9 phosphorylation. Studying the serine-9 phosphorylation of GIRK2 expression could help with understanding the reason why there was no significant change with BC treatment.

7. References

Chung, Hee Jung, et al. "Neuronal activity regulates phosphorylation-dependent surface delivery of G protein-activated inwardly rectifying potassium channels." *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 2, 2008, pp. 629-634.

Lüscher, Christian, and Paul A. Slesinger. "Emerging concepts for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease." *Nature Reviews Neuroscience*, vol. 11, no. 5, 2010, pp. 301-315.

8. Acknowledgments

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