Carnegie Mellon University

Neuroscience

G-protein Controversy

Drawing from a graduate course designed by Dr. Paul Slesinger, I have designed an undergraduate sequence of classes (3 x 50 minute class periods) and associated assignments to explore the bitter debate about whether the alpha subunit or the beta/gamma subunit of the G-protein that couples to the muscarinic potassium current in cardiac cells activates potassium channels.

This module uses a Just in Time Teaching approach in which students read and answer questions before class time. The answers to these assignments are reviewed by the instructor just before class and are used as a guide for the class time.

In this module, students first see the original data that indicates, unexpectedly, that the beta/gamma subunits signal; then they see the vicious rebuttal of that result, then they see some additional experiments done on both sides of the controversy. Students evaluate the data and arguments, learn the value of careful experiments, see science as a non-linear process, and brainstorm about additional quesitons they have and experiments to begin to address these questions.

This module was originally designed as part of a 2014-2015 Wimmer Faculty Fellowship in Teaching Innovation in collaboration with The Eberly Center for Teaching Excellence and Educational Innovation at CMU.

Module Sequence: Does the alpha or the beta/gamma subunit of G-proteins activate cardiac K+ channels?

This unit should be taught immediately after a textbook introduction to cell signaling and GPCR's. Ideally, students should have worked through a classical G-protein model. The ideal model is adrenaline because it speed heart rate (as opposed to acetylcholine which slows it down). Students should understand:

- Ligand binding
- Receptor activation and GTP exchange in the alpha subunit
- Dissociation from beta/gamma subunits
- Alpha subunit binding to adenylyl cyclase
- cAMP production
- Protein kinase A activation
- Signal amplification
- GTP hydrolysis & signal termination

In particular, students should be able to answer all of these questions [docx] before beginning.

Students should also understand the basics of recombinant DNA technology. Ideally, students should also understand the basics of active transport and Na+/K+ pumps, electrochemical gradients, and ion channels (minimally, these should not be completely foreign concepts, a deep understanding is not essential). Although an understanding of how membrane potential controls excitability is nice, it is usually beyond the scope of my course and I make do with asking students to trust me that "as positive potassium ions leave the cell, the cell becomes less active".

Homework 1

Following that introduction, students should read (as a group homework assignment) Logothetis, et al., 1987 and answer these questions [docx].

Class day 1

Recap the cannonical adrenaline model with the alpha subunit.

Discuss the homework questions and work through for students the method of isolated patch recording, measurement of currents, what a non-hydrolysable GTP analog is and how it causes persistent activation of G-proteins, and the very basic (how you would explain it to your 15 year old cousin in 5 minutes) idea of protein purification ("start with a mix, isolate the beta/gamma subunits").

Homework 2

Refer students back to Logothetis, et al., 1987 and refer students to Birnbaumer & Brown, 1987 rebuttal and ask more questions about Logothetis, et al. plus a question about Birnbaumer & Brown [docx].

Class day 2

Recap the cannonical adrenaline model with the alpha subunit.

Work back through results from Logothetis, et al. with special emphasis on how, for the first two figures, each side of the debate (alpha or beta/gamma) would explain the results. **Students should understand that figures 1 & 2 don't provide any evidence either way about which subunit is involved.** Then discuss the possibility of a low concentration of alpha subunits remaining in the "purified" beta/gamma subunits used in figure 3 of Logothetis (one of the criticisms raised by Birnbaumer & Brown). Finally, remind students of recombinant DNA technology, explain that bacteria have no endogenous G-proteins, and explain why recombinant G-proteins can therefore eliminate the possibility of contamination.

Homework 3

Students should read and compare Codina, et al., 1988 with Reuveny, et al., 1994 (sample questions here [docx]). Poll students either before class or at the start of class day 3 for what they believe is the active subunit: alpha, beta/gamma, both together, either one, neither.

Class day 3

1. Recap canonical (alpha subunit) adrenaline pathway.
2. Recap value of recombinant (as opposed to purified) G-proteins

3. Discuss key results (first couple figures each) of Yatani and Reuveny papers
4. Have students share and discuss their own experiment ideas
5. Look at class numbers from outside of (or beginning of) class
6. Reveal that beta/gamma is active subunit and recap acetylcholine/muscarinic signal from start to finish in light of that fact. (Compare to cannonical adrenaline pathway.)
7. Discuss the value of debate (see Osborne, 2010)
   - Clapham ended up being right, but his first attempt was sloppy and had some potential for error (e.g. contaminants in the purified beta/gamma subunits).
   - Birnbaumer's criticism and subsequent contradictory results prompted Clapham and others (e.g. Lily Jan) to use better techniques.
   - Even though Birnbaumer's results ended up unreproducible (sidebar: explain that this is ***bad***), his criticism prompted others to improve their science.

Ask students to brainstorm on new questions they would like to have answered.

Be prepared to discuss other systems that use this mechanism of beta/gamma activation (e.g. $GABA_B$ receptors & baclofen and their role in treating spasms and, possibly, addiction).

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