Date: Mon, 15 Sep 2003 00:26:22 +0000 (UT)
From: neurosci@natureny.com

To: steve.goldstein@yale.edu

September 14, 2003

Dear Steve,

Your manuscript "Subunit composition of Kv4.2-KChIP2 potassium channel complexes" has now been seen by three referees. In view of their comments (below), I am afraid we are unable to publish it.

Although we have no doubt that your work will be of interest to colleagues in the field, our referees have unfortunately raised some substantive concerns, which we feel preclude publication in Nature Neuroscience rather than a more specialized journal. In particular, although all the referees agree the work is technically well done, referees 2 and 3 both noted in comments to editors that the conclusions do not seem significant or surprising enough to justify high-profile publication.

Thank you in any case for the opportunity to consider this manuscript. I am sorry we cannot be more positive on this occasion, but we hope that you will soon receive a more encouraging response elsewhere, and that you will find our referees' comments helpful.

Sincerely yours,

Editor
Nature Neuroscience

http://www.nature.com/neuro/

Reviewer #1:

This is a nice and crisp study to determine the subunit stoichiometry of a potassium channel complex formed by Kv4.2 (an alpha subunit) and KChIP (a beta subunit). Using a combination of techniques, the authors demonstrate conclusively that a Kv4.2-KChIP complex has four subunits of each type. This information is valuable for fully understanding the functions of KChIP, a unique calcium-sensing beta subunit that confers and regulates a host of channel properties and has become a subject of intense studies in the last couple of years, partially because of its potential as a therapeutic target.

A drawback of this work is that the Kv4.2- KChIP complex is purified from subunits expressed in a heterologous expression system rather than from a native tissue. As the authors pointed out, Kv4 subunits interact with other beta subunits including Kv beta2 and DPPX in addition to KChIP. Thus it is unclear whether a 4:4 stoichiometry apply to a native complex if a tissue expresses multiple types of beta subunits. Admittedly, it is very difficult to obtain the subunit stoichiometry of a native channel complex and it is certainly not a prerequisite for the publication of this work.

Reviewer #2:

The paper from Kim et al. uses exquisite biochemical manipulations to determine the subunit composition of Kv4.2-KchIP2 complexes. The elegant use of an introduced CTX site permitted quantitative measurements and monitoring of the complex stability. I have only two comments. First, the study is performed using heterologously expressed recombinant channels. The authors show very clearly that the altered channels

harboring the CTX site and the 1D4 tags behave acceptably like their wild type counterparts and I'm certain that the deduced stoichiometries are correct. In this study the purification showed excess KChIP2 protein in the soluble material and flow through. Since the major message of this paper is the stoichiometry, does this situation of excess KchIP2 reflect the situation found in cardiac or neuronal tissues, or might the stoichiometry actually be different in vivo, engendering diverse properties? This point might well be noted in the discussion. Second, the discussion includes too much detail concerning an additionally submitted manuscript from this group concerning an electron micrographic study of these complexes, and here, this should be abbreviated to contain the issues salient to the present manuscript.

Minor points.
KChIP subunits do not 'enjoy' anything.

The review cited for beta subunits deals mostly with MiRPs, and better, more comprehensive reviews are available for references.

I believe that NCS-1 is the ortholog of the originally described frequenin, a point that is increasingly overlooked.

Reviewer #3:

The manuscript by Kim et al. describes the generation of a toxin sensitive and purification-tagged Kv4.2 subunit and the use of this mutant Kv4.3 subunit to express and purify Kv4 channels together with their KChIP2 accessory subunits. The major result of this paper is to confirm that, as for other Kv channels and their accessory b-subunits, the stoichiometry of the Kv4:KChIP channel complex is 1:1. Together with the data presented in the companion paper, under review elsewhere, these studies provide the first glimpse at the structure of Kv4 channels and one of their accessory subunits.

The paper is very clearly and concisely written. The approach of generating a toxin-sensitive mutant to monitor the structural integrity of the purified channel complexes is elegant and the advantages of the 1D4 purification tag have been exploited beautifully to obtain large quantities of highly pure Kv4:KChIP complex. The authors deserve considerable credit for taking on this ambitious, technically challenging project and producing results of exceptionally high quality.

My only significant question is whether the authors performed further purification, and specifically, isolated a bona-fide plasma membrane fraction by differential centrifugation, to address some of the heterogeneity in the mass of the purified KChIP:Kv4 complex. Using their detergent lysis/extraction procedure, the authors are capturing not only the mature, plasma membrane Kv4 channel pool but also Kv4:KChIP channels in various stages of biosynthesis. As formation of tetrameric, toxin binding Kv channels has been shown to occur very early in channel biosynthesis, it would be good to know if the 1:1 stoichiometry applies to the plasma membrane pool, or whether the Kv4.2 and KChIP2 stoichiometry is established early in biosynthesis (as for Kv1:Kvb) complexes and maintained for the lifetime of the channel, or whether KChIPs are added in a sequential manner, during maturation, to pre-formed Kv4 tetramers.

Minor correction: p3, Results, "great sensitively" should be great sensitivity.