

Yeasty brew yields novel calcium channel inhibitor

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The yeast two-hybrid system has proved remarkably successful in the study of protein-protein interactions. Since its inception, several modifications have been developed to enable comparable studies of the interaction of proteins with nucleic acids or small molecule ligands. In this issue, Young et al. introduce a new twist on the yeast two-hybrid that has yielded a novel small molecule inhibitor that disrupts the normal interactions between the pore-forming $\alpha 1$ subunits and regulatory β subunits of calcium channels. Ultimately, drugs based on this calcium-channel-directed mechanism could provide new treatments for neurological disease.

Calcium channels are present in all excitable cells, where their function is to mediate calcium entry in response to membrane depolarization and thereby couple electrical signals in the cell membrane to calcium-dependent intracellular processes, such as contraction, secretion, neurotransmission, and gene regulation. The L-type calcium channels in cardiac and smooth muscle are the molecular targets for the calcium antagonist drugs that are widely used in the therapy of hypertension, cardiac arrhythmias, and angina pectoris.

Although distinct N-type and P/Q-type calcium channels are responsible for neurotransmitter release at synapses in the nervous system, it has not been possible to develop comparable highly specific organic calcium channel inhibitors for these neuronal calcium channels. Major efforts have been directed at this problem because it is thought that specific block of release of the excitatory neurotransmitter glutamate would be a useful therapy for stroke, chronic pain, and other neurological disorders.

Initial purification studies of calcium channels¹ revealed the $\alpha 1$ and β subunits, which form part of a five-subunit complex² (see Fig. 1). Ten $\alpha 1$ subunit genes and four β subunit genes are now known^{3,4}. The identity of the $\alpha 1$ subunit determines the functional type of calcium channel¹. Coexpression of β subunits is required for normal expression

and function of $\alpha 1$ subunits⁵. In general, calcium channels containing β subunits are expressed at higher levels and are activated more easily⁶. β subunits bind to $\alpha 1$ subunits through a specific interaction domain⁷ (Fig. 1), providing a novel molecular target for drug design and development.

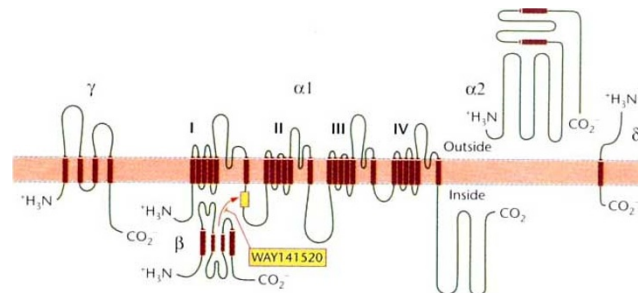


Figure 1. A novel inhibitor blocks binding of the calcium channel β subunit to its interaction domain on the $\alpha 1$ subunit. The subunits of voltage-gated calcium channels are illustrated as transmembrane folding diagrams. Cylinders represent predicted alpha helical segments. The filled rectangle represents the interaction domain for the β subunit in the intracellular loop connecting domains I and II of the $\alpha 1$ subunit. The novel compound WAY141520 inhibits β subunit binding.

Most screening methods for calcium channel inhibitors involve measurements of calcium influx through specifically expressed calcium channels in mammalian cell lines. The electrophysiological and optical methods used to measure calcium influx are difficult to implement for rapid screening of large numbers of candidate inhibitors, and the resulting drug candidates often block calcium entry nonselectively in a wide range of calcium channels and may even have effects on structurally related sodium and potassium channels. Targeting the domain responsible for regulatory interactions between $\alpha 1$ and β subunits (Fig. 1) is a novel approach that could potentially circumvent some of these problems.

Measurements of specific protein-protein interactions can be made efficiently using the yeast two-hybrid assay⁸, which measures cell growth dependent on reconstitution of a bipartite Gal-4 transcription factor by interaction of fused proteins. In the present paper, Young et al. prepare a bait protein consisting of the calcium channel $\beta 3$ subunit⁹ fused to the DNA-binding domain of Gal-4 and a prey protein consisting of the β subunit-binding segment of the $\alpha 1B$ subunit of N-type calcium channels¹⁰ fused to the activation domain of Gal-4. These two fusion proteins were coexpressed in the same yeast strain with a cycloheximide sensitivity marker whose transcription was driven by a Gal-4-responsive promoter element. In the presence of cyclo-

heximide, cell growth depends on inhibition of the interaction between the β subunit and its binding site from the $\alpha 1$ subunit of calcium channels, which prevents expression of the cycloheximide-sensitive marker protein.

Screening 156,000 compounds yielded 10 positives that supported yeast growth.

WAY141520 inhibited N-type calcium channels with a K_i of 95 μM , but did not inhibit sodium or potassium channels detectably. Its inhibition of N-type calcium currents was independent of the well-known G protein-mediated inhibition of these channels¹¹. Isolation of this compound provides proof of principle for this novel drug screen and opens the way for further use of the yeast two-hybrid assay in drug discovery.

While WAY141520 defines a novel class of calcium channel inhibitors, it is a long journey from the present compound to a useful drug. Much higher affinity, in the submicromolar range, and demonstrated selectivity among calcium channels are needed before in vivo studies of drug effects are likely to be productive. Animal studies, toxicity testing, and clinical trials loom in the future for any selective and high-affinity drug candidates that emerge from this approach. It will be of great interest to pharmacologists working on drug design and to biomedical scientists in general to watch whether this novel and convenient drug screening technology can also contribute to optimization of the affinity and selectivity of this new family of calcium channel inhibitors and eventually succeed in development of a new family of calcium channel-directed drugs of therapeutic value. In the meantime, we can all cheer as the remarkably flexible yeast two-hybrid assay finds another ecological niche for itself in biomedical science.

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