Discovering ion-channel modulators – making the electrophysiologist's life more interesting

'New fluorescence technologies can provide effective high-throughput functional screening for ion channels'

Ion channels are very attractive targets for therapeutic manipulation. Approximately 15% of the top 100 selling drugs are modulators of ion channels and the total annual worldwide sales of ion-channel modulators exceeds $5 billion. The importance of membrane voltage and ion permeability in the control of cell function ensures that modulation of ion channels will invariably have important consequences for cells and tissues, and such modulation can often be turned to therapeutic advantage. Major therapeutic indications for ion-channel modulators include cardiac arrhythmia, hypertension, anxiety, epilepsy, pain, chemotherapy-induced nausea and diabetes, as well as a range of drugs in development for important new indications including neuroprotection.

The revolution in genomics has identified a large number of new ion channels, many of which will eventually lead to the development of novel and selective therapeutic agents for major diseases. The new paradigm for drug discovery, embraced at least in part by many pharmaceutical and biotechnology companies, is to identify targets from genome databases, or by homology or functional cloning, and to couple this with additional information such as tissue expression patterns or changes in disease states. However, as is becoming very apparent to everyone in drug discovery, the identification of a novel sequence does not guarantee the development of a profitable drug. Apart from issues of target validation (which can mean anything from demonstrating therapeutic utility in the clinic to persuading senior management that a target is worthy of significant resources in discovery), there are a number of practical issues. These include obtaining the correct full-length clone, achieving adequate expression of functional channels, devising an assay for screening purposes and identifying small-molecule lead compounds to initiate medicinal chemistry.

Unlike earlier paradigms in drug discovery, this process often does not automatically provide small-molecule ligands for the targets on which medicinal chemistry lead optimization programs can be based. To overcome this, many companies have established high-throughput assays that allow extensive collections of compounds to be screened against the target to identify starting leads for novel synthesis. However, configuration of such assays can be difficult or time-consuming, and ion-channel targets have been particularly challenging in this regard.

Extremely detailed, high-value information on ion channels can be obtained using a variety of electrophysiological techniques, and these have been the gold-standard for research and drug discovery in this area for a considerable time. However, these techniques are not particularly amenable to automation and high throughput, and this has therefore been a significant limitation to either the adoption of such targets for drug discovery or has required devotion of extensive highly-skilled resources and time. To overcome these limitations, a number of technologies have been developed that allow higher throughput screening to be carried out on ion-channel targets, including ligand binding, radioactive ion fluxes, intracellular Ca^{2+} measurements coupled to membrane voltage changes, and fluorescence-based methods for

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the direct measurement of membrane potential. Each of these techniques gives different information on the interaction of compounds with ion channels and has their own advantages and disadvantages in terms of both information content and ease of automation.

Ligand binding is relatively easy to configure as long as a suitable specific ligand is available that can be radiolabeled, and a sufficient expression level of the channel can be obtained (not always easy). However, no functional data can be obtained by this method, and although separation-based binding assays are routinely used on automated systems, they are intrinsically less automation-friendly and noisier than addition-only assays. Radioactive ion-flux measurements generally give a functional readout, although they may not be simple to automate. Measurement of intracellular Ca^{2+} can be an addition-only assay, but provides an indirect readout of ion-channel activity (except of course for Ca^{2+} channels themselves), and could be subject to artifacts if the test compounds interact with Ca^{2+} channels or Ca^{2+}-release mechanisms.

Measurement of the membrane potential of a cell provides a direct functional readout that is highly dependent on ion-channel activity. However, until recently, the fluorescence-based methods for membrane-potential measurement that might be amenable to high-throughput formats have not been particularly sensitive or robust. Newer methods in this class[1] are now being developed which are robust and produce high throughput. However, while these methods have a lower informational content compared to single-cell or patch-clamp electrophysiology, they give a more direct functional measure of channel activity compared to many of the alternative high-throughput formats.

The development of improved technologies for automated high-throughput screening of ion-channel targets in drug discovery, particularly those based on highly-sensitive fluorescence read-outs, will not only allow the rapid identification of lead compounds for subsequent medicinal chemistry optimization, but will mean that specialist scientists do not have to carry out the often onerous task of screening several thousands of compounds in low-throughput formats. The new technologies can enable electrophysiologists to concentrate on high-value, high-content investigations of a small number of key ion-channel modulator leads, and as indicated in the title of this editorial, make their life much more interesting.

REFERENCE

1 Gonzalez, J.E. *et al.* (1999) *Drug Discovery Today* 4, 431–439

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