The development of cell-based assays for pain drug discovery

Since the completion of the Human Genome Project and the beginning of the so-called genomics era in the 1990s, a molecular target-based approach has been the method of choice for drug discovery. However, the landscape of drug discovery is altering once more, swaying back towards traditional physiology-based and phenotypic approaches, which have been far more effective at producing first-in-class medicines[1,2]. Specifically, phenotypic screening has been especially successful in the area of neurodegenerative diseases, representing an appealing approach in the search for drugs that acts on the complex nervous system.

By Dr Paul Karila and Dr Charlotta Blom

Before the introduction of target-based approaches to drug discovery, physiological and phenotypic approaches were used extensively in the discovery and development of new medicines. Often phenotypic studies involve little or no knowledge of the molecular mechanisms involved in a disease, and instead compounds of interest are determined based on their empirical influence on a disease phenotype.

With the completion of the Human Genome Project, it became easier to elucidate the molecular basis for a disease and identify the specific genes, proteins etc involved. This paved the way for a target-based drug discovery approach, whereby compounds are designed to specifically affect these isolated drug targets. It was thought that this method would revolutionise the process of drug discovery and development, allowing the rational design of medicines and the possibility to test their effects on a specific drug target that is directly associated with a particular disease, often using *in vitro* methods. It would seem, however, that this is not the case. Instead, a number of studies have noted a decline in drug R&D productivity and associated this with the advent of target-based drug discovery methods[3-7].

Target-based versus phenotypic drug discovery

This decrease in R&D success with target-based approaches is not so surprising given the complex nature of disease biology. There are some high-profile success stories for the target-based drug discovery approach, such as Imatinib (or Glivec) which targets and inactivates the Bcr-Abl fusion kinase responsible for causing chronic myelogenous leukaemia (CML). In this case a genetic abnormality in the Philadelphia chromosome results in the formation of a mutated fusion protein, which is active for much longer periods of time than normal, and therefore directly causes the hallmark and disease phenotype of CML – an overproduction of white blood cells[8]. It is not often the case, however, that only one protein target is implicated in causing a certain disease. Similarly, a single solution to a molecular hypothesis (eg inactivating an overactive protein) may not be sufficient or relevant to circumvent a disease phenotype.

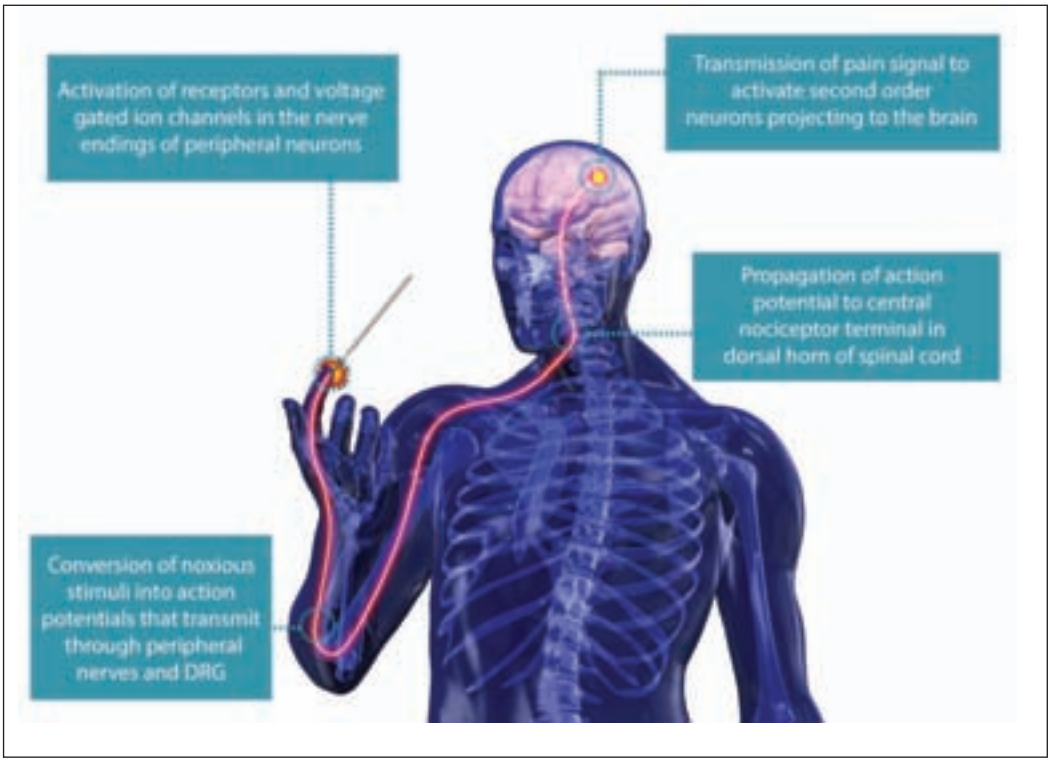

Figure 1: Pathway of pain transduction

These considerations are especially true for diseases of the nervous system, which are vastly complex and involve further intricacies.

In contrast to target-based approaches, phenotypic drug discovery requires no prior knowledge of the molecular mechanism of a disease, overcoming a major disadvantage of target-based methods. Documented success of phenotypic screening, especially in the discovery of drugs to mitigate symptoms of diseases involving the nervous system, suggests that there is still an important place for this approach in modern day drug R&D, indeed there have been numerous successes using phenotypic screening in drug discovery[1]. Notably for neurodegenerative diseases, phenotypic screening has been far more successful than the target-based approach for drug discovery, with examples including Memantine for the treatment of mild to moderate Alzheimer's disease[9] and Varenicline (or Chantix) for nicotine addiction. However, progress in phenotypic drug discovery for the nervous system is still relatively slow in comparison to other disease areas such as infectious and metabolic diseases, where accurate and predictive phenotypic assays are readily available. In areas such as the nervous system, including diseases associated with ageing and pain, screening models are more limited or lacking. Promising advances are emerging, however, namely the development of cell-based assays for nervous system and pain drug discovery, which are set to provide new opportunities in the search for novel medicines that target neurodegenerative diseases and chronic pain.

Overview of pain mechanisms

Chronic pain is a major public health problem, estimated to increase with the constantly growing world population. There are a number of analgesic compounds already available, which, although they do have therapeutic utility in some pain states, are ineffective in between one and two-thirds of patients suffering from chronic pain conditions[10,11]. These drugs also suffer from drawbacks in clinical use, with common side-effects including neurotoxicity, dependence and gastrointestinal effects such as constipation[12]. There is therefore a significant and unmet need for improved analgesic compounds, either based on novel or existing mechanisms.

Acute (or physiological) pain is the result of intense and/or damaging stimuli, often termed noxious stimuli. The detection and transmission of noxious stimuli is a vital physiological mechanism that provides protection from injury. Chronic (or persistent) pain, however, is a condition where the

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pain sensation lasts beyond the term of an injury, and generally a long time after the noxious stimulus has disappeared or the injury has healed.

Pain transduction[13] occurs through the activation of specific receptors and voltage-gated ion channels in the nerve endings of peripheral nerve cells (neurons). These neurons at the injury site convert noxious stimuli into electrical signals (action potentials), which are propagated to the central nociceptor terminal located in the dorsal horn of the spinal cord. From here, transmission of the pain signal occurs, activating second-order neurons that project to the brain.

There are a number of added complexities to this pain transmission process, with rapid functional changes and long-term regulatory changes at both the peripheral and central levels of the nervous system. The perception of pain is also subject to a process called sensitisation[14], which leads to an increase in the responsiveness of neurons and is a key process in chronic pain states[15]. As a result, neuronal excitation and pain sensation is achieved with stimuli of lower intensity than normal, while neurons may also exhibit an enhanced response to noxious stimuli. An important molecular mechanism for peripheral pain sensitisation appears to be through altered neurotrophic signalling, whereby an increase in the concentration of agents such as nerve growth factor (NGF) results in a hypersensitivity to heat and mechanical stimuli[16].

This complex system thus represents an opportunity for drug R&D; to discover and develop compounds that are able to address the problems and symptoms caused when these pain mechanisms become damaged in some way.

Challenges in finding phenotypic screening methods

One major obstacle in the search for drugs for the treatment of chronic pain, is a lack of appropriate phenotypic assays and model systems, although examples are emerging of cell-based phenotypic assays. However, a number of challenges must be overcome in the design of a phenotypic assay which allows the study of nervous system function at several levels of complexity – from the intact organism to the molecular level. Importantly, relevance to the intact organism must be maintained while also striving to achieve sufficient throughput to enable the testing of larger compound sets. A well-defined phenotype is also essential, as understanding the phenotype of a model system and how it is affected by specific diseases makes the design of assays, as well as the interpretation of screening results, much easier and more reliable. Cell-based models that represent the full physiological properties of neurons and neuronal networks are highly desirable to enable higher throughput testing in the search for new drug candidates[17].

Primary neurons as model systems

Primary neurons, specifically Dorsal Root Ganglia (DRG) cultures, represent an attractive system for the study of cellular and molecular mechanisms of sensory neuron function. Postnatal and adult DRG cultures also offer the possibility of studying mature neurons with characteristics that resemble the *in vivo* complexity of the peripheral nervous system. Indeed, these neurons have been used successfully in a number of studies[18-20]. Through these studies, DRG sensory neuronal cultures have been shown to retain their sensory functionality *in vitro*, where they are able to respond to thermal, mechanical and chemical stimuli. Importantly, it is also possible to generate an *in vitro* model of peripheral sensitisation, a hallmark of chronic pain, by exposing the neurons to NGF.

One consideration for the use of primary DRG cultures in phenotypic screening assays is cell supply. Compared to immortalised cell lines, a popular choice for cell-based assays, DRG's cannot be expanded into desired quantities and so it is more difficult to test larger compound libraries. High sensitivity assay technologies capable of handling reduced cell volumes must therefore be employed when primary neurons are used in screening.

Phenotypic assay readout

When designing a phenotypic, cell-based assay, an important consideration is which characteristic will be used as a phenotypic readout or endpoint to the assay. Survival or neuronal death is often used, however, viability may not accurately reflect neuronal dysfunction in, for example, neurodegenerative diseases. Functional synaptic changes and neurite structural changes may occur much earlier than cell death. Consequently, more useful readouts for nervous system disease screens are morphological changes such as neurite length or number and also functional readouts such as neuronal excitability and synaptic function.

A functional phenotypic approach enables the identification of compound effects, irrespective of mechanism of action, increasing the likelihood of picking up compounds with relevant activities. It is the assays that monitor functional aspects of neuronal networks, which are becoming increasingly more appealing in the discovery and development of new medicines for diseases and conditions affecting the nervous system[21].

Alterations of neuronal excitability and synaptic function are hallmark events in a number of diseases affecting the nervous system and therefore provide the ideal readouts for phenotypic assays that study pain and diseases of the nervous system. Assays that enable the identification of compounds affecting such events are therefore highly desired.

Screening for neuronal excitability

Neuronal excitability is an especially important assay readout for studying chronic pain. In neurons, voltage-gated sodium channels are essential to generate and propagate an electrical signal, with the excitability of a peripheral sensory neuron dictated by the type of channels present, as well as their expression levels and plasma membrane distribution[22]. These sodium channels are produced in the cell body and transported over relatively large distances to their site of action. Mutations that affect this trafficking process, leading to changes in neuronal excitability, have been shown to underlie some major diseases and also play a role in chronic pain[23]. In addition, the expression and function of ion channels are also influenced by the release of inflammatory mediators, such as NGF, which is a key manifestation of chronic pain[24].

Voltage-gated sodium channels, therefore, offer a desirable target for the design of drugs that target the pain pathway. However, although this has been known for a number of years, there are still no approved drugs available to selectively target these channels, suggesting that target-based drug discovery approaches may have failed thus far. This means there is an opportunity of employing phenotypic screening methods that assess alterations of neuronal excitability in DRG sensory neurons, which may prove to be more promising in the search for chronic pain medicines.

Phenotypic screening in excitable cell cultures

As mentioned, one of the major challenges in phenotypic screening is to maintain relevance to the intact organism while also achieving a high throughput. Realising both of these will result in a robust strategy for phenotypic screening, and in terms of the nervous system, the key is to be able to test neuronal excitability, network activity and plasticity in high-throughput assays. In addition, a high level of expertise is required in this complex and specific area of research in order to design and analyse the results of phenotypic screening in the nervous system.

To this end, a cell-based assay has been developed

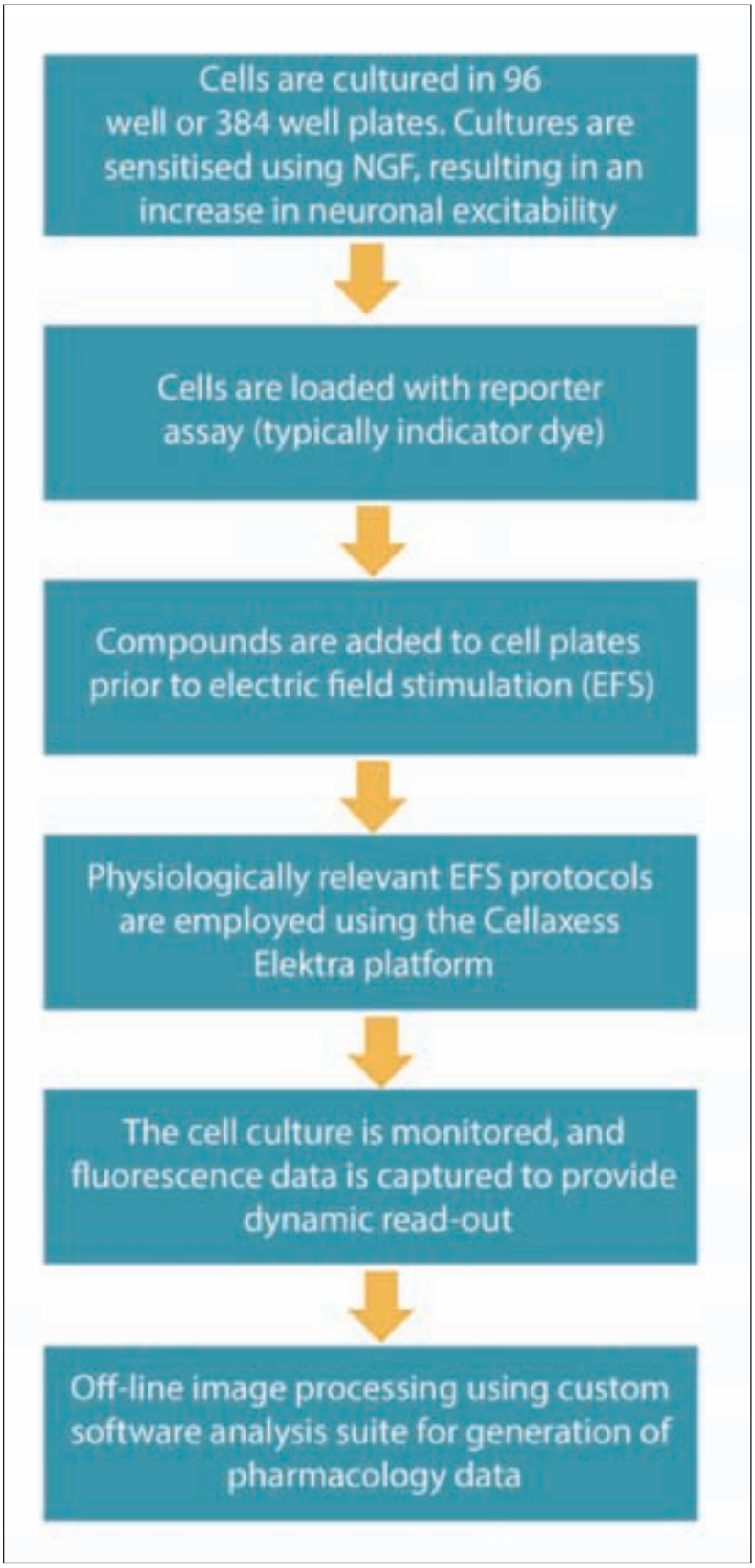

Figure 2: A cell-based assay for pain drug discovery

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by Cellectricon that combines its in-house expertise and proprietary technology with the aim of identifying novel targets related to pain. It combines electrical field stimulation (EFS), to trigger neuronal activity, with plate-based imaging to monitor excitability changes in adult rodent DRG neurons. High-content analysis is possible as it enables *in situ* manipulation and monitoring of neuronal cell cultures directly. Initially the DRG cultures are sensitised using NGF, which manifests as an increase in the neuronal excitability. EFS is then applied to achieve physiologically relevant excitation of the cultured neurons. By applying an external electrical field to a neuronal culture, the cellular transmembrane potential is altered, causing inward currents through voltage-gated sodium channels. The response can then be measured using imaging techniques, whereby neurons are stained with a fluorescent indicator.

Through this platform the neuronal response to EFS is investigated and the excitability and synaptic function monitored[25]. Changes in the magnitude of these responses as a result of the application of different compounds in a screen will identify those compounds likely to have a positive impact on the treatment of chronic pain. Throughout the assay, cell viability and morphology is maintained and a high level of throughput is possible – overcoming the key challenges associated with designing assays for phenotypic screening.

Another example of a recently established cell-based assay that applies to the nervous system is being used to study Alzheimer's disease[26]. In this case the biogenesis and synaptic action of amyloid-beta (A) peptide allows for the screening of clinical compounds that alter A levels in a mouse model of Alzheimer's disease.

Conclusions

While phenotypic screening approaches are again rising in popularity in the drug discovery business, there is a need for robust and efficient assays that enable the study of the nervous system. Phenotypic assays can be highly predictive for infectious and metabolic diseases, however, for more complex disease areas such as the nervous system and cancer, more effort needs to be placed in designing sufficient and accurate models and assays. Cell-based assays are now emerging, such as for pain sensitisation and also in the screening of clinical compounds to treat Alzheimer's disease. It is hoped that the further development of these cell-based assays will open the door for phenotypic screening approaches in nervous system and pain drug discovery. DDW

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