It’s all in your head

Renowned pharmacologist Dr Raymond Dingledine shares his insight into the field, as well as explaining his translational work towards a greater understanding, and improved treatment, of epilepsy and brain injury.

You are currently researching the translational benefits of understanding the responses of the brain to injury and prolonged seizure. What is your motivation for this research?

Epilepsy and traumatic or ischaemic brain injury together affect over 8 million patients in the US alone, carrying enormous societal and personal cost. Preventing epilepsy for those at risk, and blunting the brain damage associated with stroke and traumatic brain injury, are strong unmet medical needs worldwide. Traumatic brain injury is the signature hidden wound of our soldiers in the Middle East, and is also being recognised as an outcome risk for some of our more aggressive contact sports. Our work aims to develop novel ways to blunt brain inflammation, in order to prevent or modify the intensity of these brain disorders. Our goal is to improve the lives of patients afflicted by such disorders.

What skills and experience do you bring to this project?

My original training was in biochemistry, pharmacology and electrophysiology, and over the years we have incorporated a host of additional approaches and methodologies into our work. I have come to an appreciation of what is needed to move a bench discovery to the marketplace – and thus to patients – by co-founding the biotech start-up NeuOp Inc., serving on the joint steering committee of a NeuOp project with Bristol-Myers Squibb, serving on the scientific advisory board of a philanthropic venture called The Epilepsy Therapy Project, and helping a large pharmaceutical company in some patent litigation cases. I also gained some administrative and financial experience by becoming Chair of Pharmacology and Research Dean at the Emory University School of Medicine, serving as Editor of the scientific journal Molecular Pharmacology, chairing the Investment Committee of the Society for Neuroscience, and chairing the Program Advisory Committee of the Morehouse Neuroscience Institute. These activities have all been enormously rewarding on a personal level as well.

How important is a multidisciplinary, collaborative approach to your research?

A multidisciplinary approach has been essential to the success of our research. It would not have been possible to even start our current work without the expertise and energy brought to the project by the team at our Emory Chemical Biology Discovery Center, who coordinated the high-throughput screening and guided the secondary screening that ultimately resulted in convincing identification of a competitive EP2 antagonist. Iterative efforts of the neuroscientists and a medical chemist in our laboratory were necessary to improve the structure of our library lead compound and to carry out the animal tests. Beyond the team efforts within our own laboratory at Emory, we have benefited tremendously from interactions with personnel from the US Food and Drug Administration, the wisdom of other laboratories nationally that are associated with the CounterAct project, and discussions with numerous colleagues in the global epilepsy community.

At what stage is your project currently? How do you hope to progress with your research in the future?

We are currently using medicinal chemistry to improve the aqueous solubility and selectivity of our lead EP2 receptor compound. We now have compounds that exhibit more than 500 times higher selectivity for EP2 than other prostanoid receptors and compounds that are 10 times more soluble than our original research lead. We are using these compounds in our own laboratory to explore the effects of anti-inflammatory treatment in animal models of Alzheimer’s disease and epilepsy-related depression, and collaboratively in several other disorders that have a prominent inflammatory component. We don’t expect EP2 antagonists to be a panacea, so it will be important to search for biomarkers that can predict responsiveness to anti-inflammatory therapies.

Might your research contribute to a better understanding of other conditions or diseases?

Our major effort is with epilepsy. However, inflammation driven by cyclooxygenase and its downstream pathways contributes to numerous other diseases and neurological disorders including stroke, cystic fibrosis, inflammatory bowel disease, some cancers and major depression. Our EP2 antagonist project might offer promise in these and similar indications.

You have tremendous experience both as a researcher and as a scientist, and your career has seen notable successes. What practices have helped you to achieve this?

I’m reminded most days that no man is an island. I have been very fortunate throughout my career to work with an outstanding group of students, postdocs and other colleagues. It has been gratifying and very rewarding to work with them and watch their careers flourish.
Seizing the opportunity

THROUGHOUT ITS HISTORY, epilepsy has been a much misunderstood illness. Ancient cultures such as the Greeks, Romans and Babylonians viewed the condition as a form of spiritual malady akin to possession, and documented cases in Ayurvedic and Akkadian texts demonstrate a similar understanding. In 400 BCE, Hippocrates attempted to refute this idea, announcing to the world that epilepsy was not a divine illness, but rather the product of a problem with the brain; he was widely disbelieved. It was not until the 19th Century that the affliction was distinguished from extreme forms of mental illness, and the first anti-seizure medication, bromide, was produced. Today, epilepsy affects around 1 per cent of the global populace, with more than 3 million patients with the condition in the US alone.

Thankfully, 60-70 per cent of epilepsy cases in developed nations can be controlled with current medications, allowing many to live with the disease. Because of this, and the relatively widespread nature of the illness, epilepsy is a fairly well-known medical condition – but the same cannot be said of status epilepticus. This severe and often life-threatening condition is defined as a seizure lasting longer than 30 minutes – after which time the danger of permanent damage to neurons and the blood-brain barrier is greatly increased. Roughly one in five of these seizures is fatal, and as such status epilepticus is always treated as a medical emergency. About 40 people in every 100,000 in the US, and only one-quarter of patients are epileptic.

A WAY TO GO

Although understanding of seizures and epilepsy has improved markedly since ancient times to some extent the condition still lives up to the vague name it bears: epilepsy, Greek for ‘to afflict’. The molecular mechanisms responsible for the initiation of seizures, or epileptogenesis, are still poorly understood.

In order to develop new anti-seizure drugs and, more importantly, anti-epileptogenic drugs that interrupt or retard the progression of the disease, much research attention has been devoted to elucidating these complex causative interactions. In recent years, this study has focused particularly on inflammation – a factor which, in some instances, has led to an increased insight into the disease, as well as potential new routes toward treatment.

One particular group at Emory University in the US has been responsible for pushing research in this area a long way forward over the last few years. Dr Raymond Dingledine leads a laboratory in the Department of Pharmacology at the University, and is also the co-founder of the Emory Chemical Biology Discovery Center. Over the last three years, Dingledine and his collaborators have been investigating a number of inflammation pathways and their involvement in the precipitation of seizures; their results have often been surprising, and present a number of possible routes forward for clinical practice as well as basic research.

INITIAL INVESTIGATION

These novel investigations began several years ago when, after deciding to take a fresh look at the challenge of preventing or mitigating the effects of epilepsy, the researchers noticed an intriguing trend in their lab results and the results of others. It emerged that the enzyme cyclooxygenase 2 (COX-2) is rapidly induced by seizures, and is responsible for producing five different prostanooid molecules including prostaglandin E2 (PGE2) – which in turn activates four receptors: EP1, 2, 3 and 4. EP2 appeared to have a pro-inflammatory effect on peripheral tissues when activated, and since the Emory scientists already knew that inflammation could play a role in the onset of seizures and other neurological disorders, this was the final piece of the puzzle.

The Georgia team therefore elected to focus specifically on EP2, with their initial experiments aiming to inhibit the EP2 receptor and determine the effect of this inhibition on seizure-related inflammation and its consequences. In order to achieve this, however, the researchers had to overcome a problem that for others might have been insurmountable: EP2 antagonists were not commercially or privately available at that time. The solution reached by Dingledine and his collaborators was straightforward although not simple: since there were no EP2 antagonists available, they would make their own. First, they developed cell lines that expressed human EP2 receptors, then they developed a cell-based assay to detect EP2 inhibitors, and finally they used the assay to screen 262,000 compound samples from their small molecule library. This approach was very successful – the group discovered 13 molecules that could act as high-affinity competitive inhibitors of EP2.

MAKING PROGRESS

Focusing on one of these molecules in particular, the researchers then initiated a brief medicinal chemistry campaign to improve its brain penetration and half-life in plasma; once this was achieved, they then had a lead compound to begin proof-of-principle animal studies. These experiments showed that the chosen EP2 inhibitor brought about a range of beneficial effects in three separate models of status epilepticus, when administered in divided doses beginning 80 minutes to four hours after
seizure onset. Not only did the compound prove to be anti-inflammatory as well as neuroprotective, but it also prevented seizure-induced opening of the blood-brain barrier. In addition, the substance greatly reduced delayed mortality after a bout of status epilepticus, and accelerated the rodents’ recovery of weight and normal nest building behaviour.

The conclusion of Dingledine and his lab colleagues, therefore, was that EP2 inhibition, initiated an hour or more after seizures begin, could reduce the mortality and morbidity associated with status epilepticus – and that this boon might be just as helpful to humans as it had proven to mice. Going forward, the group is pursuing microglia, a prominent cellular target for the compound’s anti-inflammatory effects, and is delving even deeper into the inflammatory pathways themselves, elucidating the mechanisms by which the compound achieves its success. As with many successful projects, the brains behind this work are very impressive – but in this case, the number of brains that could be protected by it is even more so.

A CENTRE FOR DISCOVERY

The researchers in Dr Raymond Dingledine’s team at Emory University have many tools at their disposal, but one particular advantage they enjoy is access to the Emory Chemical Biology Discovery Center (ECBDC). Dingledine was a co-founder of the ECBDC when it was established 10 years ago, with the goal of expanding collaboration between the University’s department of chemistry and school of medicine. The multidisciplinary centre has two missions: firstly, to discover novel chemical leads targeted to disease-related proteins; and secondly, to train the next generation of drug discovery scientists. In the pursuit of these goals, the ECBDC also plays an important role in accelerating the process of translational work.

In 2004 the Center became one of the founding members of the National Molecular Libraries Screening Center Network, and through this partnership the ECBDC scientists have carried out numerous projects from labs throughout the nation that involve assay development and high-throughput screening. The ECBDC is now under the leadership of Drs Haian Fu and Yuhong Du, who have added their special expertise in small molecules targeted to protein-protein interactions, which is an emerging focus in drug discovery.