"New Frontiers in Epilepsy"

Despite the fact that epilepsy is the commonest neurological disorder with therapeutic indications, having a prevalence of between 0.5-1% of the population, it is only in recent years that there has been a concerted effort to provide more rationally designed and selective drugs for the treatment of this disorder. Many advances in understanding the biochemical and structural abnormalities that underly seizure disorders have been made, and the genetic aetiology of the epilepsies is in the process of being unravelled. The latest trends in research into epilepsy were described at a one-day symposium "New Frontiers in the Treatment of Epilepsy" organised by the Society for Medicines Research. The meeting was held on 17th April 1997 at SmithKline Beecham's impressive "New Frontiers Science Park" in Harlow, UK.

David Chadwick (Walton Centre for Neurology, Liverpool) discussed many of the current issues in the treatment of epilepsy. Epilepsy is a group of disorders in which seizures occur and not a homogeneous disease entity. Seizures may occur as (i) acute symptomatic seizures, (e.g. due to alcohol abuse); (ii) isolated cryptogenic seizures; or (iii) epilepsies (remote symptomatic, genetic, cryptogenic). Any kind of cerebral trauma can give rise to epilepsy and for approximately 70% of cases, no cause can be clearly identified. However, it is believed that at least 30-40% of these cases are genetic in origin. Seizures can be classified into two types: localised or partial which are preceded by 'aura', and generalised with no warning, leading to loss of consciousness and a tonic/clonic seizure. Approximately 50% of seizures are of the partial type and the majority of these arise in the temporal lobe of the brain. By classifying epilepsy syndromes (seizure type, age of onset, EEG evidence, associated impairments), clinicians can begin to rationalise their approach and define the therapeutic options for each patient. When to start and/or stop drug treatment in epilepsy is a major issue, which requires detailed knowledge of the prognosis of the disorder. For 20-30% of sufferers, epilepsy is a chronic and disabling condition, refractory to drug treatment, which has immense social impact. Although currently available drugs are able to prevent seizures, there remains a clear unmet medical need for new antiepileptic drugs. Chadwick went on to outline his criteria for the ideal antiepileptic drug (AED) which should have:-

- A clearly identified and novel mechanism of action;
- Simple pharmacokinetic profile - no interactions with existing drugs;
- Efficacy across the broad spectrum of seizure types;
- Low toxicity and wide therapeutic window;
- Low cost.
The currently-available range of AED's were then compared with the above criteria. Many of these drugs interact with the metabolism of other AED's and can produce unwanted side-effects or toxicity. In spite of recent advances in AED's and the introduction of new drugs such as Lamotrigine, Topirimate and Gabapentin, Professor Chadwick concluded that there was still a great deal of room for improvement in the current drugs available for the treatment of epilepsy.

Patients with refractory partial seizures may be candidates for surgical treatment, and the second speaker, John Duncan (National Hospital for Neurology and Neurosurgery, London), clearly described the application of methods of structural and functional neuroimaging to the treatment of epilepsy. Magnetic resonance imaging (MRI) has been applied to the investigation of epilepsy for the past 12 years. The principle role of MRI is in the definition of structural abnormalities that underly seizure disorders. MRI represents a major advance over CT scanning - for example, in 100 adults with chronic partial epilepsy 94% of CT scans will be normal, whereas 85% of MRI's will identify some structural abnormality. In patients with intractable temporal lobe epilepsy, MRI can reliably identify hippocampal sclerosis, which is the commonest cause of partial epilepsy, and surgery can render 60-70% of these cases seizure free. It is also possible to use MRI to calculate the volume of the hippocampus by imaging 1mm sections and show whether the damage is occurring in a specific site (e.g., the anterior region) or is diffuse. Using 3D imaging, the surface of the cortex can be reconstructed and a range of malformations of cortical development identified. Functional MRI is sensitive to the oxygenation state of haemoglobin and shows changes in regional blood volume and flow. The data obtained is analogous to that from PET studies and can be acquired at the time of seizures. Using MRI it is possible to identify the cerebral areas that are responsible for specific cognitive processes, allowing surgeons to accurately plan resections close to eloquent cortical areas.

Magnetic resonance spectroscopy (MRS) provides a non-invasive means of investigating cerebral metabolites and some neurotransmitters. Proton MRS can be used to estimate the concentrations of N-acetyl-aspartate (NAA), creatine and choline containing compounds. A reduction in the ratio of NAA to creatine + choline is a feature of cerebral regions that include epileptic foci, and this method can be of value in identifying focal abnormalities which are not visible using MRI. Changes in the cerebral concentrations of GABA, glutamine and glycine can also be estimated using MRS, aiding the identification of different epileptic syndromes and prescription of the most suitable drugs.

Positron emission tomography (PET) has been used to provide data on regional cerebral blood flow (rCBF), glucose metabolism and the binding of specific ligands to receptors. An epileptic focus is commonly characterised by an area of
reduced glucose metabolism, identified using $^{18}$F-fluorodeoxyglucose ($^{18}$FDG), and a smaller area of reduced benzodiazepine binding, identified using $^{11}$C-flumazenil ($^{11}$C-FMZ). Again, these techniques compliment the use of MRI. Single photon emission computed tomography (SPECT) can also be used to produce images reflecting rCBF. Although a particular strength of SPECT is the ability to obtain images related to rCBF at the time of seizures, caution is required when interpreting the data. Duncan concluded by summarising the many advances in structural and functional imaging in epilepsy over the past 12 years, noting that the proportion of cryptogenic cases will decrease with future improvements in hardware, signal acquisition techniques and post-processing methodologies.

Serendipity has been the cornerstone of AED discovery for the greater part of the 20th century. In his lecture, Graeme Sills (Epilepsy Unit, Western Infirmary, Glasgow) detailed the push towards rational drug design. To put things in perspective, 30% of epilepsies are currently uncontrolled. There is little rational basis for use of AEDs and side effects and drug interactions are major problems. Current developments in mechanism based drug design hopefully will overcome these issues and allow for more confidence in therapy. To aid these mechanistic approaches, understanding the pathophysiology of epilepsy is important. Better animal models are also needed but the two main ones which are most useful are MES, maximal electroshock induced seizures in mice, and PTZ, pentazoline induced seizures in mice. In neurochemical terms, seizures have as a root cause, inhibition of the glutamate (excitatory) or reduction of the GABA (inhibitory) neurotransmitter systems. Thus, clear targets for interaction are their relevant ion channels (NMDA, AMPA for example), neurotransmitter uptake systems, such as the GABA transporter, and neurotransmitter metabolic pathways. In the past eight years several new drugs have emerged. These were described in some detail highlighting what is known of their mechanisms of action, and their effectiveness in animal models. This information will give clearer guidance for future areas of research. Vigabatrin (1989) is a GABA analogue and GABA transaminase inhibitor. It is ineffective against MES and PTZ models. Seizure control is better when given chronically. Lamotrigine (1991) is a sodium channel blocker effective against MES. Felbamate (1993) is a GABA A receptor agonist and NMDA antagonist effective in MES and PTZ models. Gabapentin is another GABA analogue with an unknown mechanism of action, but may involve calcium and sodium channels. It is also effective in MES and PTZ models. Topirimate (1995) has a sugar-based structure and has multiple mechanistic actions. In neurochemical terms, it has no effect on the level of GABA or glutamate. Tiagabine was the subject of another lecture (see below). Remacemide (Phase
IIII is another sodium channel blocker effective in the MES model. Oxcarbazepine works mainly by sodium channel blockade and is effective against MES. It is metabolised to a monohydroxy derivative, in common with carbamazepine (CBZ). CBZ however reaches this endpoint via a biologically active epoxide. Oxcarbazepine may therefore have fewer unwanted side effects. The mechanism of action of levetiracetam is unknown but, after 60 minutes of exposure, it gives rise to increased striatum levels of GABA. Losigamone blocks sodium channels and GABA A receptors and is effective in the MES and PTZ models.

Developmental areas were discussed and were classified in four categories: excitatory amino acids, calcium channel blockers, GABA compounds and purinergics. These areas embrace many mechanistic targets. Suggestions for improved therapy included NMDA antagonists, glycine site antagonists, kynurenic acid, L-type calcium channel blockers and sub-unit specific GABAergic compounds. Sills concluded that, as our ability to dissect and disseminate the biochemistry and pathophysiology of epilepsy expands, then it is likely that so too will the AED armamentarium.

In an attempt to gain an understanding of epilepsy that has a genetic predisposition, John Dailey (Univ of Illinois College of Medicine, Peoria, USA) discussed progress using animal models. Both genetic and induced seizure models have utility in drug development but Dailey considered that the genetic models have been under-utilised in the past. There are many genetic epilepsy animal models known, for example, the genetically absence epilepsy rat (GAER), genetically epilepsy-prone rat (GEPR), various mutant mice (including DBA/2, E1, quaking, tottering, lurcher and staggerer), Mongolian gerbil, epileptic baboon and several more. GAER and GEPR in particular have many desirable characteristics. They mimic human seizures and the human response to drugs. The seizures are predictable and reproducible. Ease of handling is also important. The GAERs model EEG and behavioural aspects of human absence seizures, a form of non-convulsive epilepsy. In neurochemical terms, the spike and wave discharges are facilitated by noradrenergic and dopaminergic decrements and GABAergic increments. Seizures are suppressed by ethosuximide and other anti-absence drugs, but exacerbated by phenytoin and other anticonvulsant drugs.

In contrast, GEPRs model three types of convulsive epilepsy: generalised tonic-clonic, partial, and partial with secondary generalisation to tonic-clonic. The GEPRs have a predisposition to sound induced seizure represented in terms of an audiogenic response score (ARS), which ranges from 0 (no response) to 9 (severe seizure). Thus, a GEPR rat exhibits a characteristic convulsive response to each ARS. GEPR 3s have moderate seizures exhibiting clonic convulsions whilst
GEPR 9s exhibit complete tonic extension. Anticonvulsant treatment can lower the ARS score. GEPRs respond to broad spectrum drugs, and also to those useful in generalised tonic/clonic and partial seizures, but not baclofen and chlorpromazine. In neurochemical terms there is a clear deficiency of noradrenaline, serotonin (5-HT) and probably GABA, and possibly an excess of glutamate/aspartate.

Dailey has used these genetic models to research the connection between 5-HT and AEDs. There is evidence that 5-HT is anticonvulsant, so he asked the question “Do AEDs release 5-HT?” Microdialysis experiments were used to measure extracellular levels of 5-HT in the hippocampus of freely moving rats. Carbamazepine (4xED50) increased 5-HT levels seven fold in GEPR 9 rats. Zonisamide, valproate, loreclazole and antiepileptirine have all been shown to release 5-HT. There is an increase in 5-HT levels from administering the SSRI antidepressant drug fluoxetine. This coincided with an ARS decrease. Prior 5-HT depletion decreases the anticonvulsant effectiveness of drugs which release 5-HT but does not decrease the anticonvulsant effectiveness of drugs which do not release 5-HT. Dailey clearly felt that new anti-epileptic drug development should address serotinergic systems.

Lars Knutsen (Novo Nordisk, Denmark) recounted the history of the discovery of Tiagabine (Gabitril) and then went on to give details of its development and early marketing. Novo Nordisk are co-developing this product with Abbott. Tiagabine was first synthesised in 1986 specifically as an uptake inhibitor of GABA. It is now known to be a selective inhibitor of GAT1 (one of four subtypes of GABA transporters) which is located solely in the CNS. The starting point for the drug development programme was SKF 89976, a lipophilic nipecotic acid derivative known to cross the blood brain barrier. Structure activity relationships were described and these have been published elsewhere. Tiagabine was selected on a combination of factors: its symmetry, log P (a measure of lipophilicity), in vitro potency and selectivity, and in vivo effects. In vivo it is most potent in DMCM (mice) and PTZ(rat) induced seizure models which are both GABAergic mechanism based. Chronically, there are no signs of withdrawal, no tolerance to the anticonvulsant activity, no abuse potential, no clinically significant organ related toxicity and no teratogenic, mutagenic or carcinogenic effects. In man, it has linear absorption rising to a peak at 90 minutes, 89% bioavailability, and a plasma t1/2 of 7-9 hours which can be reduced to 2 -3 hours if liver enzymes are induced by other drugs. Over 2,000 patients participated in long term clinical trials. Over 1000 patients have received the drug for 1 year. Gabitiril is indicated for add-on therapy, partial seizures with and without secondary generalisations, in males and females over 12 years old,
and was first launched in Denmark in November 1996. Further launches have followed in Austria, Switzerland and Germany, with launches in France and the UK planned for 1997.

Following on from Professor Chadwick's requirement for novel AED's to possess a novel mechanism of action, Neil Upton (SmithKline Beecham, Harlow), described the discovery and pre-clinical pharmacology of SB-204269 (trans-(+)-6-acetyl-4S-(4-fluorobenzoylamino)-3,4-dihydro-2,2-dimethyl-2H-benzo[b]pyran-3R-ol). SB-204269 was developed starting from the observation that K⁺ conductances are intimately involved in the inhibition of neuronal excitability and cell firing. The group at SB therefore proposed that drugs which stimulate the opening of K⁺ channels should diminish neuronal excitability. Experiments with the prototypical K_ATP channel opener, cromakalim, demonstrated that this compound could inhibit seizures in a variety of rodent models. A series of analogues of cromakalim was examined for anticonvulsant activity following p.o. administration in the mouse maximal electroshock seizure threshold (MEST) test and improved activity was observed when the pyrrolidinone ring at the 4-position in the cromakalim molecule was replaced with a 4-fluorobenzamide group. The team at SB then investigated the effect of changing the stereochemistry at the 3- and 4-positions on anticonvulsant and antihypertensive activity in this series of benzamides. It was shown that the anticonvulsant activity resided mainly in the 3R, 4S enantiomer, whereas the 3S, 4R enantiomer was responsible for the antihypertensive effect. Further investigation of the structure-activity relationships in the series led to the replacement of the 6-cyano group with a 6-acetyl group, resulting in SB-204269. The compound has been shown to be an orally-effective anticonvulsant agent, at doses (0.1-30 mg/kg) devoid of overt behavioural depressant properties, in rat models of both electrically and chemically evoked tonic extension seizures. SB-204269 also selectively reduces focal electrographic seizure activity in an in vitro elevated K⁺ rat hippocampal slice model at concentrations (0.1-10 µM) that have no effect on normal synaptic activity and neuronal excitability. SB-204269 was found to be inactive in a wide range of binding and functional assays, leading the team to conclude that they had a compound with a novel mechanism of action. Using ³H labelled SB-204269 they went on to demonstrate the presence of a stereoselective binding site in rat forebrain. The K_D of SB-204269 (50 nM) for this binding site was shown to correspond to the effective brain concentration of the compound in the rat MEST test and for a range of analogues there was a positive correlation between affinity for the binding site and activity in the anticonvulsant test. The pharmacological specificity of the binding site was...
evaluated using over 150 known agents, none of which had an IC$_{50}$ lower than 10 µM. The nature of this unique binding site is currently the subject of intense investigation at SmithKline Beecham. In conclusion, Upton stated that the overall efficacy profile of SB-204269, together with its minimal liability for inducing neurological impairment and an apparently unique mechanism of action involving a selective interaction at a novel binding site present in the CNS, suggested potential therapeutic utility for the compound in the treatment of refractory partial and generalised tonic-clonic seizures.

With the current intensity of gene research gripping the worldwide pharmaceutical industry, it was fitting that the meeting should close with a lecture that picked up the genetic theme again, this time focusing on humans. The area was reviewed by Mark Gardiner (Department of Paediatrics, University College, London) who took a forward looking viewpoint to assess future prospects for epilepsy treatment. A genetic aetiology is present in up to 40% of patients with epilepsy, and one way of conveniently categorising these genetic epilepsies is by their mendelian and non-mendelian patterns of inheritance. There are over 120 mendelian single gene disorders which include epilepsy in the phenotype, but these are rare (about 1%). The non-mendelian epilepsies are the common familial syndromes which display ‘complex’ inheritance patterns.

A further useful distinction is to segregate epilepsies into those which can be described as either ‘symptomatic’, in which there is a detectable structural and metabolic abnormality, or those which are ‘idiopathic’ wherein no abnormality is detectable and patients are otherwise cognitively and neurologically intact. Most progress has been made in the analysis of the mendelian patterned disorders and the gene map of the autosomal epilepsies (dominant and recessive) is steadily growing with at least 14 loci identified. For example, ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy, has been mapped to chromosome region 20q13.2 with a positional candidate gene identified in an Australian study. The gene product was identified as CHRNA4, the $\alpha_4$ subunit of the neuronal nicotinic acetylcholine receptor. EPM1, progressive myoclonus epilepsy, which is autosomal recessive, has been mapped to 21q22 with the gene product identified as cystatin B.

Human epilepsy genes can also be broadly classified as follows: genes involved in a structural/developmental role, e.g. EMX2; genes associated with progressive neurodegeneration e.g. EPM1; genes directly linked with functional ion channels that are thus involved in neuronal excitability; and those which have a metabolic involvement, mtDNA.

Evidence is accumulating that a proportion of human idiopathic inherited epilepsies are ion channel diseases where mutations in the ion channels have been
shown to cause paroxysmal disturbances of excitable tissue, such as skeletal muscle, cardiac tissue and nerves. Two classes of ion channels are implicated:
a) the neuronal nicotinic acetylcholine receptor, where we have evidence for mutations in the $\alpha_4$ subunit (CHRNA4) in ADNFLE, and the $\alpha_7$ subunit (CHRNA7) in juvenile myoclonic epilepsy (JME), and
b) voltage sensitive calcium channels, where the evidence has come from genetic mouse models, for example, the tottering mouse has mutations in an $\alpha_{1a}$ subunit which gives rise to ataxia and seizures. There is an homologous disease in humans.

Our genetic and structural knowledge of ion channels is steadily increasing, as is our knowledge of their functional role. Gardiner clearly sees these as exploitable targets for future AEDs. Future prospects also look good in the diagnostic area with a DNA based classification. Gene therapy may also be a possibility if the issues of targeting can be overcome.