Recent advances in the treatment of acute neurodegeneration

Neurodegenerative disorders constitute a major cause of death and disablement, yet no neuroprotective drug has been marketed. At a conference entitled *Recent Advances in the Treatment of Acute Neurodegeneration*, organized by the Society of Chemical Industry Fine Chemicals Group in London in May this year, a number of different approaches and new developments for the treatment of the acute brain injury caused by events such as ischaemic stroke and severe head injury were discussed.

Excitatory amino acids

There is compelling evidence that injury to the brain initiates a cascade of processes that, over a period of hours to days, causes additional damage. Dr Alan Palmer (Cerebrus, Ascot, UK) reviewed evidence that supports a pivotal role for the excitatory amino acids (EAAs), glutamate and aspartate, in the neurodegenerative changes associated with acute brain injury. EAAs act on *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate and metabotropic receptors. Most excitatory transmission is mediated through AMPA/kainate receptors because the NMDA receptor is quiescent under normal circumstances as a result of a depolarization block by Mg^{2+} ions. It is now clear that EAA-induced toxicity is mediated primarily by a massive influx of Ca^{2+} ions through the NMDA-receptor-gated channel, a process that is inhibited by competitive NMDA-receptor antagonists (for example CGS 19755, Ciba-Geigy) and noncompetitive antagonists (for example Cerestat, Cambridge Neuroscience) that block the channel. Although such compounds show clear neuroprotective efficacy in a variety of animal models, clinical development has been hampered by the occurrence of unwanted side effects (for example hypertension, neuronal vacuolization, psychomimetic effects and memory impairments).

NMDA receptor subtypes

NMDA receptors are assembled from distinct subunits: NR1 in combination with NR2A, NR2B, NR2C and NR2D. While NR1 subunits show a ubiquitous brain distribution, NR2 subunits display regional selectivity with NR2B subunits located predominantly in the forebrain and NR2C occurring mainly in the cerebellum. This regional variation provides an opportunity for the development of an antagonist for a single NMDA receptor subtype, which might possess fewer side effects than existing non-subtype-selective antagonists.

Dr Bert Chenard (Pfizer, Groton, CT, USA) presented data that suggested that ifenprodil (**1**), an antagonist of the polyamine site of the NMDA receptor, acts selectively at the NR2B subunit found predominantly in forebrain regions. By a process of methodical structural change, CP 101,606 (**2**) was identified as having a greater potency and selectivity for the NR2B subunit than the original lead structure. CP 101,606, which has proceeded into clinical development, was shown to:

- protect cultured hippocampal neurons (but not cerebellar granule cells) from glutamate-induced toxicity,
- improve behavioural outcome following experimental brain trauma and
- attenuate trauma-induced elevations in intracranial pressure.

AMPA/kainate-receptor antagonists

The activation of AMPA/kainate receptors contributes to excitotoxic injury in a number of ways, including an increase in the production of reactive oxygen species (probably via metabolic stress caused by sustained neuronal depolarization) and an increase in cytosolic concentrations of free Ca^{2+} by both direct mechanisms (some AMPA/kainate receptors gate Ca^{2+} ions) and indirect mechanisms (via voltage-operated Ca^{2+} channels and the NMDA receptor, following removal of the depolarization block). Antagonists of the AMPA/kainate receptors therefore have great potential as neuroprotective agents. The first generation of AMPA/kainate-receptor antagonists, compounds such as NBQX (**3**) and related quinoxalinediones, were highly efficacious in animal models of both focal and global ischaemia, but did not progress to the clinical development stage because of their poor water solubility, poor brain penetrability and nephrotoxicity. Dr Jorgen Drejer (Neurosearch, Glostrup, Denmark) presented data on a new generation of AMPA/kainate receptor antagonists, the isatin-oximes (an example is NS 420, **4**), which have good water solubility, a long duration of action, an improved side-effect profile, and receptor-subtype selectivity.

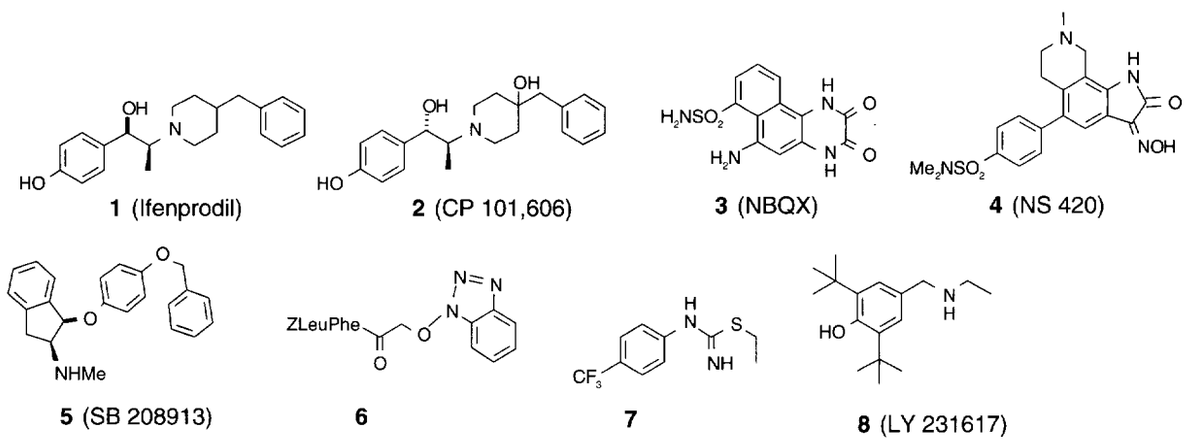
Subtype-selective mGluR ligands

In addition to the ionotropic receptors, metabotropic glutamate receptors (mGluRs) may also mediate excitotoxicity. The latter receptors have been divided into three subgroups based on their sequence homologies, signal transduction mechanisms and pharmacology:

- subgroup I (mGlu1 and mGlu5) is coupled to phosphoinositide hydrolysis and is selectively activated by quisqualate and (1*S*,3*R*)-1-amino-cyclopentane-1,3-dicarboxylic acid;
- subgroup II (mGluR2 and mGluR3) is negatively coupled to adenylate cyclase and is selectively activated by (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV);
- subgroup III (mGluR4, mGluR6, mGluR7 and mGluR8) is also coupled to adenylate cyclase, but is selectively activated by L-2-amino-4-phosphonobutanoic acid (L-AP4).

Dr David Jane (University of Bristol, UK) reviewed recent advances in the development of subtype-selective mGluR ligands developed from phenylglycine and α-substituted analogues of glutamate.

  1359-6446/96/$15.00. PII:S1359-6446(96)30081-0



*Some potential neuroprotective agents. These compounds illustrate the variety of targets that can be employed to attenuate the cascade of neurodegeneration that follows acute brain injury. The forebrain-selective NMDA-receptor antagonist CP 101,606 (**2**) was developed from ifenprodil (**1**), and the AMPA/kainate receptor antagonist NS 420 (**4**) from NBQX (**3**). Blockade of these receptors diminishes the injury-induced elevations in the concentrations of cytosolic free Ca^{2+}, as do inhibitors of voltage-operated Ca^{2+} channels such as SB 208913 (**5**). Excitotoxic mechanisms can also be blocked by inhibiting processes downstream of EAA receptors. Thus inhibition of the activity of calpain and nNOS (e.g. with **6** and **7**, respectively) confers neuroprotection. Finally, inhibition of the neural injury by antioxidant molecules such as LY 231617 (**8**) provides an additional approach to the development of an effective therapy for ischaemic stroke and severe head injury.*

Some of the compounds had neuroprotective efficacy: the presynaptic mGluR antagonist (*S*)-4-carboxy-4-hydroxyphenylglycine diminished striatal lesions induced by quinolinic acid; the combined mGluR1 antagonist and mGluR2 agonist DCG-IV protected against both NMDA and kainic-acid-induced toxicity; and L-AP4 diminished the toxicity caused by nitric oxide in hippocampal neurones. There is clearly much untapped potential in the utility of mGluR ligands as neuroprotective agents.

Voltage-operated calcium channels

Elevations in the cytosolic concentration of free Ca^{2+} ions derive not only from Ca^{2+} influx through the NMDA-receptor-channel complex, but also from Ca^{2+} entry into the cell through voltage-operated Ca^{2+} channels (VOCCs). Blocking these channels provides an attractive strategy for neuroprotection. Pharmacological studies have divided VOCCs into four subtypes: L, N, P and Q. Dr Barry Orlek (SmithKline Beecham, Harlow, UK) described the development of the indanamine class of calcium channel antagonists, which were neuroprotective in gerbil models of global cerebral ischaemia. In addition, SB 208913 (**5**) produced insignificant cardiovascular side effects, which might reflect a selective action on N rather than L channels.

Intracellular enzymes

A further opportunity for the development of novel therapies for acute brain injury is provided by the inhibition of biological processes downstream from EAA receptors and VOCCs. A number of ways of attenuating the effects of elevated cytosolic concentrations of free Ca^{2+} were discussed. Dr Ron Bihovsky (Cephalon, West Chester, PA, USA) described the development of novel inhibitors of calpain I, a subclass of Ca^{2+}-activated thiol proteinases, which act on cytoskeletal proteins such as spectrin, microtubule associated protein (MAP), tubulin and actin. Using human recombinant calpain I as a screening tool, several potent calpain inhibitors were developed (for example benzotriazolyloxymethyl ketone **6**), which have neuroprotective properties in a gerbil model of cerebral ischaemia.

The development of isoform-selective inhibitors of nitric oxide synthase (NOS) was discussed by Dr Martin Drysdale (GlaxoWellcome, Stevenage, UK). Nitric oxide is involved in a number of important physiological and pathological processes. The endothelial isoform of NOS (eNOS) regulates blood pressure and blood flow. In the CNS, the predominant neuronal isoform (nNOS) is implicated in cerebral ischaemia and is regulated by cytosolic levels of free Ca^{2+}. Nonselective inhibitors of NOS are neuroprotective *in vitro* and *in vivo*, even though eNOS inhibitors could potentially increase neuronal damage through vasoconstriction. Selective nNOS inhibitors can therefore be expected to provide a greater degree of neuroprotection than nonselective NOS inhibitors. Using recombinant enzymes, a series of isothioureas, including compound **7**, were developed as selective nNOS inhibitors, which may have potential as neuroprotective drugs.

In addition to Ca^{2+}-mediated toxicity, EAAs also cause the production of reactive

oxygen species (ROS), which may exacerbate excitotoxicity by inhibiting EAA uptake. ROS are also formed by a number of other mechanisms following brain injury. Potent antioxidant molecules therefore have clear neuroprotective potential. Dr Jill Ann Panetta (Lilly, Indianapolis, IN, USA) illustrated this by describing the discovery of benzylamine LY 231617 (**8**), which inhibits Fe-induced lipid peroxidation and also diminishes H_2O_2-induced loss of cultured hippocampal cells and global ischaemia-induced loss of cells from the CA_1 region of the hippocampus.

Prospects for the future

In his summing up, Dr Ian Cliffe (Cerebrus, Ascot, UK) emphasized the fact that the prospects for the development of effective treatments for severe brain injury appeared good, even though the issues involved were multifactorial and complex. Drugs that block the cascade of destruction associated with excitotoxic injury appear to have the greatest potential. Preclinical studies have already established the utility of NMDA and AMPA/kainate receptor antagonists in the treatment of acute brain injury, and a number of NMDA receptor antagonists (e.g. Cerestat) are now in clinical development for ischaemic stroke and severe head injury. It seems only a matter of time before an EAA-receptor antagonist with an acceptable side-effect profile is launched on the market. The likelihood of this occurring has been increased by the emergence of subsite-selective EAA-receptor antagonists. Prospects for effective therapy have been further enhanced by the discovery of inhibitors of excitotoxic processes downstream from EAA receptors and the development of neuroprotective antioxidants. Dr Cliffe concluded that, although the emergence of effective therapies for chronic neurodegenerative disorders (for example Alzheimer's disease, Parkinson's disease and motor neurone disease) appear to be somewhat further in the future, it is clear that the same approaches may provide treatments for both acute and chronic neurodegenerative disorders. Thus the prospects for effective neuroprotective therapies for chronic neurodegenerative disorders will be substantially improved by the introduction of well-tolerated neuroprotective drugs to treat acute brain injury.

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Combinatorial chemistry and automation

In June, 150 international scientists, managers and decision makers from the pharmaceutical industry, research institutes and universities gathered in Geneva, Switzerland for IBC's *Third European Forum on Combinatorial Chemistry & Automation: Applications for Accelerated Drug Discovery.*

Combinatorial chemistry is probably one of the most actively expanding research areas within the pharmaceutical industry, and the application of combinatorial chemistry to facilitate and accelerate drug discovery has, from the beginning, been totally dominated by US scientists, despite the fact that some of the pioneering work underlying combinatorial chemistry had both Australian and European roots. An early (but often overlooked) European contribution to combinatorial chemistry came from Dr Pierre Chambon's group in Strasbourg on multi-oligonucleotide synthesis on cellulose discs[1]. At approximately the same time, Dr Mario Geysen and his Australian coworkers presented their work on multipeptide synthesis on pins[2]. Later, the group of Dr A. Furka in Hungary pioneered the 'split-and-mix' synthesis scheme[3,4].

The conference presented some of the celebrities of combinatorial synthesis as keynote speakers, including Drs Eric Martin and Jeff Jacobs (formerly Affymax Research Institute, CA, USA; both currently at Versicor, South San Fransisco, CA, USA) as well as Dr Wolfgang Rapp (Rapp Polymere, Tübingen, Germany), the inventor of the renowned and widely used TentaGel™. Thus, the scene was set for a successful conference – especially so because merging of the fields of automation and combinatorial chemistry remains one of the most important tasks for the successful implementation of high-throughput synthesis in the pharmaceutical industry.

Single compounds

It is always difficult to select the highlights from such a meeting, but this report summarizes some of the new trends. One such trend is the tendency to favor single compounds, especially so in the major pharmaceutical industry. The fact that European pharmaceutical industry is picking up the concept of combinatorial synthesis was illustrated by Dr Gabriele Handke (Bayer AG, Leverkusen, Germany). She presented the solid-phase synthesis of a series of quinazolines, which is quite a common pharmacophore. The use of a relatively simple set-up enabled the synthesis of 96 different quinazolines as single compounds in 10–20 mg quantities over the course of two days. Another point that was brought up was the theme of diversity. Dr Handke took a rather pragmatic view, defining a chemical library as diverse if it would bind to or activate more than one biological target.

1359-6446/96/$15.00