Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man

JAMES McCULLOCH

Wellcome Surgical Institute and Hugh Fraser Neuroscience Laboratories, University of Glasgow, Glasgow G61 1QH

- 1 A wide range of therapeutic strategies has been explored in humans and experimental animals with the aim of improving outcome after brain ischaemia but few have shown convincing clinical benefit.
- 2 The massive increase in the extracellular concentration of glutamate which occurs in cerebral ischaemia is a key component in the sequence of neurochemical events which leads to neuronal death. Pharmacological blockade of the action of glutamate at the *N*-methyl-D-aspartate (NMDA) receptor, (the glutamate receptor subtype principally involved in the neurotoxic effects of the amino acid) provides a novel therapeutic approach to cerebral ischaemia.
- 3 The effects of NMDA receptor antagonists in animal models of focal cerebral ischaemia are uniquely consistent, *viz*, a marked reduction in the amount of irreversible ischaemic damage irrespective of the species, the model of cerebral ischaemia, when the animals are sacrificed after the ischaemic episode, whether ischaemia is permanent or temporary and followed by reperfusion and which particular NMDA antagonist was employed.
- 4 NMDA receptor antagonists have marked effects on brain function in normal animals. The balance between these potential adverse effects and the anti-ischaemic efficacy of these drugs will ultimately determine the clinical utility of this class of drugs.
- 5 The data which are reviewed provide the basis for the current clinical evaluation of NMDA receptor antagonists in stroke and head trauma.

Keywords *N*-methyl-D-aspartate ischaemic brain damage glutamate receptor

Introduction

The importance of cerebral ischaemia is a reflection of the frequency of cerebrovascular disease in advanced societies and the severity of its sequelae. Cerebrovascular disease ranks third (after cancer and heart disease) as the cause of death in Western Europe and North America and is the major cause of handicap in the adult population. Approximately 500,000 people in the U.K. are presently incapacitated by the neurological effects of cerebral ischaemia.

Focal cerebral ischaemic damage (stroke) results from a reduction in cerebral blood flow to a discrete brain area. The origin of the ischaemic episode may be occlusive (due to *in situ* arterial thrombosis), embolic or haemorrhagic. In some patients it is due to a combination of proximal vascular narrowing and impairment of total cerebral blood flow, e.g. due to a sudden reduction in

cardiac output. Ischaemic brain damage is a feature of a number of clinical conditions other than stroke, most notably head injury, prolonged seizures, cardiac arrest, perinatal hypoxia, etc. These conditions provide additional patients who may benefit from excitatory amino acid receptor antagonists and, as in the case of head injury, clinical populations in which the efficacy and potential adverse reactions of these class of drugs may be readily studied.

Stroke therapy can be directed at a wide range of pathophysiological mechanisms and there has long been particular interest in medical and surgical therapies designed to improve cerebral blood flow to the ischaemic tissue. Drugs which putatively increase flow to ischaemic tissue, such as nimodipine, are of clear benefit to subarachnoid haemorrhage patients who are at high risk of

Correspondence: Professor J. McCulloch, Wellcome Surgical Institute and Hugh Fraser Neuroscience Laboratories, University of Glasgow, Garscube Estate, Bearsden Road, Glasgow G61 1QH

This paper is based on a presentation at a symposium on 'Cerebral vascular disease – new therapeutic opportunities', sponsored by the Clinical Section of the British Pharmacological Society, held at the University of Glasgow in July 1991.

delayed ischaemia due to vasospasm and reduced cerebral blood flow (Pickard *et al.*, 1989). Nimodipine may also be of benefit in stroke, but the evidence is more controversial (Gelmers *et al.*, 1988). In addition, there has long been concern that simply increasing blood flow to ischaemic brain tissue may have adverse consequences such as increased cerebral oedema, haemorrhagic transformation or generation of free radicals (Hossmann, 1982).

The role of excitatory amino acids in the genesis of ischaemic neuronal injury

The concept that blockade of excitatory amino acid receptors attenuates the transmembrane ionic fluxes that lead to neuronal death provides a therapeutic strategy that does not depend upon improvement in cerebral blood flow. High concentrations of glutamate are neurotoxic (Choi, 1991; Lucas & Newhouse, 1957; Rothman & Olney, 1986). From extensive investigations in cell cultures (for review see Choi, 1991), the neurotoxic effects of glutamate appear to be mediated predominantly via activation of the *N*-methyl-D-aspartate (NMDA) receptor subtype although the contribution of non-NMDA receptors is becoming increasingly recognised (see Choi, 1991; Choi *et al.*, 1988; Frandsen *et al.*, 1989; Michaels & Rothman, 1990). Recent evidence suggests that the generation of nitric oxide via NMDA receptor activation may contribute to neuronal damage (Dawson *et al.*, 1991).

In experimental cerebral ischaemia, there is a marked, immediate increase in the extracellular concentrations of glutamate and aspartate, irrespective of the nature and primary cause of the ischaemic episode (Figure 1). Ischaemia induced elevations in excitatory amino acids occur in all brain areas which have been investigated and in response to all experimental approaches employed to

provide low levels of cerebral blood flow (i.e. global ischaemia, middle cerebral artery occlusion, CNS trauma, subdural haemorrhage) (McCulloch *et al.*, 1991). The elevation in extracellular glutamate in ischaemia is due to an increased release from neurones, to an impaired uptake of glutamate into neurons and astrocytes in the ischaemic tissue and to reversal of the uptake mechanism (Nicholls & Attwell, 1990). The relationship between extracellular glutamate and cerebral blood flow is a threshold type relationship with elevation in glutamate being triggered by blood flow reduction below 20 ml $100\text{ g}^{-1}\text{ min}^{-1}$ (Shimada *et al.*, 1989), suggesting that glutamate threatens cerebral tissue in the ischaemic penumbra as well as in the ischaemic core. The blood flow threshold for irreversible damage to neurones is time dependent. Cerebral blood flow of 17 ml of blood 100 g^{-1} of brain tissue min^{-1} (or 35% of basal levels of cerebral blood flow) must be sustained for 3 h or more to produce damage, whereas neuronal damage occurs if there is a complete cessation of cerebral blood flow beyond a few minutes (Jones *et al.*, 1981).

The actions of excitatory amino acids such as aspartate and glutamate are mediated by at least four distinct receptor subtypes. NMDA, kainate and 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors have been defined in terms of their selective affinity for the appropriate agonists and these glutamate receptor subtypes are all associated with receptor operated ion channels. A fourth glutamate receptor subtype ('the metabotropic receptor') has been identified recently and is linked to phosphoinositide metabolism (Lodge & Collingridge, 1990).

There are a number of distinct sites within the NMDA receptor ion channel complex at which drugs may act to attenuate the effects of glutamate (Figure 2) (see Foster & Fagg, 1987). Conceptually, the most simple site at which NMDA antagonists can exert their action is the neurotransmitter recognition site for glutamate and NMDA, the most potent of these competitive NMDA

Figure 1 Extracellular glutamate concentrations are elevated in focal cerebral ischaemia produced by middle cerebral artery occlusion (a) and after induced subdural haematoma (b). Data are from microdialysis probes in the rat cerebral cortex. Dialysates were collected in 20 min fractions ($2.5\text{ }\mu\text{l min}^{-1}$). After middle cerebral artery occlusion, there is approximately a 20-fold increase at peak in extracellular glutamate concentrations; after subdural haematoma, there is a five-fold increase. Redrawn from the data of Butcher *et al.* (1990) and Bullock *et al.* (1991).

Figure 2 Diagrammatic representation of the NMDA receptor complex. Blockade of the NMDA receptor can be achieved at multiple, pharmacologically distinct sites. Competitive NMDA antagonists (e.g. D-CPPene, CGS 19755) act at the agonist recognition site. Non-competitive NMDA antagonists (e.g. MK-801, CNS 1102) and Mg^{++} act at distinct sites within the ion channel. Blockade of the actions of NMDA can also be achieved via blockade of the glycine recognition site (e.g. with HA-966) or polyamine site (e.g. with ifenprodil). Redrawn from Foster & Fagg (1987).

antagonists which have been studied in models of ischaemia being *cis*-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS 19755) and D-3(2-carboxypiperazin-4-yl) propenyl-1-phosphonic acid (D-CPPene) (Aebischer *et al.*, 1989; Lehmann *et al.*, 1988). Agents such as MK-801, CNS 1102 and phencyclidine (PCP) interact with a site within the ion channel of the NMDA receptor to produce a non-competitive blockade of the actions of glutamate (Kemp *et al.*, 1987). Agents such as 7-chlorokynurenic acid and 3-amino-1-hydroxy-2-pyrrolidone [(+)-HA 966] appear to attenuate the effects of NMDA receptor agonists by acting at a site through which glycine allosterically enhances NMDA receptor function (Kemp *et al.*, 1988; Singh *et al.*, 1990). Other allosteric sites within the NMDA receptor (the 'polyamine site') may be involved in the action of ifenprodil and related compounds to the NMDA receptor complex (Carter *et al.*, 1989). The opening of the NMDA receptor-ion channel is voltage-dependent by virtue of blockade with physiological concentrations of magnesium; membrane depolarisation at the onset of cerebral ischaemia relieves the magnesium block of the NMDA ion channel.

The existence of multiple, pharmacologically active sites within the NMDA receptor ion channel complex is not of esoteric neuropharmacological interest. The different sites within the NMDA receptor complex at which non-competitive antagonists (such as MK-801) and competitive antagonists (such as D-CPPene) act, and the influence of glutamate upon their interactions with their specific binding sites may have a crucial bearing on the efficacy of these two types of NMDA antagonists in cerebral ischaemia and their potential for adverse

effects on CNS function. Non-competitive antagonists such as MK-801 produce a use-dependent blockade, in which the binding of the drug to its recognition site in the ion channel and the resulting NMDA blockade are markedly enhanced by high concentrations of glutamate (Kemp *et al.*, 1987; Wong *et al.*, 1986). In contrast, the NMDA receptor blockade produced by competitive antagonists such as D-CPPene can be overcome or reduced by increasing concentrations of glutamate (Kemp *et al.*, 1987). In cerebral ischaemia the presence of high extracellular glutamate levels should intensify the blockade produced by non-competitive NMDA antagonists such as MK-801, but could potentially counteract the blockade produced by competitive antagonists such as D-CPPene.

Anti-ischaemic efficacy of NMDA receptor antagonists in experimental animals

Focal cerebral ischaemia

The effects of NMDA receptor antagonists in experimental models of focal cerebral ischaemia can be readily summarised, *viz.* these drugs effect a marked reduction in the amount of irreversible ischaemic damage irrespective of the species, the model of cerebral ischaemia, when the animals are sacrificed after the ischaemic episode, whether ischaemia is permanent or temporary and followed by reperfusion, and irrespective of the particular site within the NMDA receptor at which the drug acts.

The consistency of view which has emerged from the

use of NMDA antagonists in experimental focal ischaemia is unique for any pharmacological class of anti-ischaemic drug. The anti-ischaemic efficacy of NMDA antagonists in experimental focal ischaemia (as distinct from global ischaemia, *vide infra*) is not due to the focality of the ischaemic insult but to its moderate severity as distinct from the complete (or near complete) absence of blood flow to the brain in most reliable global models. In middle cerebral artery occlusion models of focal ischaemia, the failure of NMDA antagonists to protect the basal ganglia has been attributed to the much lower levels of blood flow which occur after occlusion of the middle cerebral artery in the caudate nucleus relative to the cerebral cortex. The lack of protection in the caudate nucleus indicates that a minimal level of cerebral blood flow is required for anti-ischaemic efficacy of NMDA antagonists (McCulloch *et al.*, 1991).

Cat The clearest evidence of the potency of NMDA antagonists as anti-ischaemic agents has emerged from studies of their effects in permanent middle cerebral artery (MCA) occlusion in the cat where the volume of ischaemic damage has been comprehensively assessed (Figure 3). Pretreatment with a competitive antagonist (D-CPPene) or a non-competitive antagonist (MK-801) or polyamine site blockers (ifenprodil and *d*-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidine ethanol (SL 82.0715)), administered within 5 min of the occlusion, markedly reduces the volume or irreversible ischaemic brain damage in the cerebral hemisphere (Figure 4) (Bullock *et al.*, 1990; Chen *et al.*, 1991; Gotti *et al.*, 1988; Ozyurt *et al.*, Uematsu *et al.*, 1991).

A critical issue for all potential anti-ischaemic com-

Figure 4 NMDA receptor antagonists markedly reduce the volume of ischaemic brain damage which results from permanent middle cerebral artery (MCA) occlusion. The magnitude of neuroprotection is similar with competitive blockade (D-CPPene, 15 mg kg⁻¹, i.v. 15 min before MCA occlusion), ion channel blockade (MK-801 5 mg kg⁻¹, i.v. 120 min after MCA occlusion), and polyamine site blockade (ifenprodil, 16.7 µg kg⁻¹ min⁻¹, i.v. initiated 5 min after MCA occlusion). Data are presented as mean ± s.e. mean (*n* = 6–13 per group). □ vehicle, ■ drug. Original data are from Chen *et al.* (1991), Gotti *et al.* (1988) and Park *et al.* (1988). Reproduced from McCulloch & Iversen (1991) with permission.

pounds is that of how long after the onset of the ischaemic episode these agents are able to prevent ischaemic damage occurring. It is self-evident that for a drug to be effective it must be present in the ischaemic tissue in adequate concentration during the time window of the therapeutic

Figure 3 Effect of MK-801 upon ischaemic brain damage after middle cerebral artery occlusion in the cat: volumetric assessment of ischaemic brain damage. a) The areas of ischaemic brain damage (solid black) assessed with light microscopy, are charted onto line drawings for 16 predetermined coronal planes. b) Effect of MK-801 (5 mg kg⁻¹ 30 min prior to MCA occlusion) on the area of ischaemic damage in the 16 coronal planes. There are significant differences between vehicle (●) and MK-801 (○) treatment at each coronal plane. Data are mean ± s.e. mean (*n* = 9 in each group). The volumes of ischaemic damage calculated from the areas and the known stereotactic co-ordinates were vehicle 3231 ± 394 mm³ and MK-801 1602 ± 445 mm³ (*P* < 0.01). Original data from Ozyurt *et al.* (1988) reproduced from McCulloch *et al.* (1991) with permission.

opportunity (i.e. less than 3 h in the cat MCA occlusion model (even with penumbra level of blood flow). The chemistry of the drug has considerable bearing on what extent (and how quickly) plasma drug levels are reflected in ischaemic cerebral tissue. For a highly lipophilic agent such as MK-801 with rapid CNS entry, the low levels of blood flow in ischaemic tissue only slightly delay its appearance in ischaemic tissue (e.g. 5 min after administration, the level in ischaemic tissue is 50% of that in the cerebellum) (Wallace *et al.*, 1992). By virtue of its rapid brain uptake, MK-801 first administered 2 h after the onset of ischaemia is as effective as pretreatment in reducing the volume of ischaemic brain damage in the cat MCA occlusion model (Park *et al.*, 1988b). In contrast for hydrophilic molecules such as D-CPPene (and almost all other competitive NMDA antagonists presently available) the rate at which equilibrium is achieved between plasma and CNS is extremely slow (half-time of CNS uptake of 60 min or more). The slow diffusion across the blood-brain barrier probably accounts for the lack of a significant effect of D-CPPene when treatment is initiated 1 h after MCA occlusion (Chen *et al.*, 1991).

There is evidence which suggests that the magnitude of neuroprotection offered by MK-801 is broadly similar in temporary MCA occlusion in the cat (2 h occlusion followed by 4 h reperfusion) (Uematsu *et al.*, 1991) to that observed with 6 h of permanent MCA occlusion (Ozyurt *et al.*, 1988; Park *et al.*, 1988a). Furthermore, nimodipine treatment together with MK-801 appears to result in greater reductions in the amount of brain damage than does MK-801 alone in the cat focal ischaemia – reperfusion model (Uematsu *et al.*, 1991).

Primate In the single study available at present, post-ischaemic treatment with MK-801 reduces the amount of brain damage and improves neurological outcome after temporary focal ischaemia in non-human primates (Zabramski *et al.*, 1991).

Rabbit The investigations of the efficacy of NMDA antagonists in focal cerebral ischaemia in rabbits, though numerically limited, contain a number of interesting features. They provide one of the few reliable demonstrations of the efficacy of MK-801 in a model of embolic stroke (Kochhar *et al.*, 1988). Functional recovery after MK-801 treatment in spinal cord ischaemia was first shown in the rabbit (Kochhar *et al.*, 1988). Dextromethorphan and its active metabolite, dextrorphan, which are weak, non-competitive NMDA antagonists, have been most extensively examined in a rabbit model of temporary focal ischaemia followed by reperfusion. Both these agents, with pretreatment and with treatment initiated at the start of reperfusion after 1 h of ischaemia, reduce the amount of ischaemic damage (assessed with histology), the amount of oedema (assessed with MRI) and improve functional recovery (assessed with somatosensory evoked responses) (George *et al.*, 1988; Steinberg *et al.*, 1988, 1991). It should be emphasised that the threshold anti-ischaemic dose of these agents in the rabbit is 15 mg kg⁻¹ (i.v.) in the first hour of treatment (Steinberg *et al.*, 1991), compared with the antitussive dose in man of 0.2–0.4 mg kg⁻¹ by mouth (3–4 times daily).

Rat The efficacy of NMDA antagonists in rat models of focal cerebral ischaemia has been confirmed in numerous reports. There is overwhelming evidence that non-competitive NMDA antagonists (MK-801, TCP, PCP) reduce the amount of ischaemic damage after MCA occlusion in the rat (Bielenberg & Beck, 1991; Dirnagl *et al.*, 1990; Gill *et al.*, 1991; Gotti *et al.*, 1988; Park *et al.*, 1988a; Roussel *et al.*, 1992). There is growing evidence for the view that competitive NMDA antagonists, glycine antagonists, polyamine site antagonists and the systemic administration of Mg⁺⁺ are also effective in focal ischaemia in the rat (Gill *et al.*, 1991; Gotti *et al.*, 1988; Izumi *et al.*, 1991; Park *et al.*, 1991; Simon & Shiraishi, 1990). The volume of tissue which can be salvaged from irreversible ischaemic damage with NMDA antagonists is approximately 50% of the infarction volume in untreated rats; the maximum anti-ischaemic effects of the drugs are broadly similar irrespective of their precise site of action within the NMDA receptor complex. Marked neuroprotection with MK-801 and PCP is observed despite the marked hypotension which it produces in halothane-anaesthetised rats (Bielenberg & Beck, 1991; Park *et al.*, 1988a). Hypotension would tend to exacerbate damage by reducing blood flow in the ischaemic penumbra to even lower levels (Osborne *et al.*, 1987). Drug-induced hypotension is the probable cause of the U-shaped dose-response curve noted with PCP and MK-801 in the rat MCA occlusion model (Bielenberg & Beck, 1991; Gill *et al.*, 1991). There is evidence (see Dirnagl *et al.*, 1990; Roussel *et al.*, 1990, 1992) that the magnitude of response to MK-801 and kynurenate may be somewhat smaller in spontaneously hypertensive animals probably because the ischaemic insult after MCA occlusion is more severe in the hypertensive strain than in normotensive animals (Roussel *et al.*, 1992).

A recent report indicates that blockade of glutamate receptors other than the NMDA subtype with NBQX can also reduce ischaemic damage in the rat (Gill *et al.*, 1992).

Perinatal hypoxia

Perinatal hypoxia, like focal cerebral ischaemia, is another area where there is convincing evidence of reductions in ischaemic brain damage. MK-801, kynurenate and dextromethorphan all putatively reduce the amount of damage produced by hypoxia and unilateral carotid artery occlusion in neonatal rats (Andiné *et al.*, 1988; Hattori *et al.*, 1989; McDonald *et al.*, 1987; Olney *et al.*, 1989; Prince & Feesser, 1988). MK-801 treatment is of benefit even when initiated up to 75 min after the hypoxic episode (Hattori *et al.*, 1989; McDonald *et al.*, 1989). Despite their undoubted efficacy in neonatal models of hypoxia, the medico-legal problems associated with administration of new drugs to brain damaged infants effectively preclude the use of NMDA antagonists in this clinical area at present (particularly within litigious North America).

Global cerebral ischaemia

The pivotal investigations on anti-ischaemic efficacy of selective NMDA antagonists were that MK-801 could

protect the hippocampus of the gerbil from the effects of global ischaemia (Gill *et al.*, 1987). The present status of NMDA receptor antagonists in animal models of global ischaemia can be summarised readily. In all of the studies of global ischaemia in large animals (dogs, cats and primates) no benefit has been demonstrated. In studies of global ischaemia in rodents (rats and gerbils), while the balance of evidence, in numerical terms, favours the view that NMDA antagonists reduce delayed damage to the hippocampus, all positive reports attract criticisms that the benefit observed is indirect (i.e. due to anti-convulsant effects, drug-induced hypothermia). The severity of the ischaemia between different models of global ischaemia appears to provide the best explanation for divergent observations between different investigations (McCulloch *et al.*, 1991).

Only MK-801 has been systematically studied in large animal models of global ischaemia. In dogs, MK-801 fails to alter neurological deficits and the amount of hippocampal damage produced by 11 min global ischaemia (occlusion of ascending aorta) (Michenfelder *et al.*, 1989) or a model of prolonged (17 min) cardiac arrest with a variety of treatment paradigms (Sterz *et al.*, 1989). Similarly, in cats, MK-801 does not improve outcome (neurological deficit and neuropathology in the cortex, hippocampus and cerebellum) after 18 min cardiac arrest (Fleischer *et al.*, 1989). In a study of non-human primates with 17 min of ischaemia, MK-801 again did not provide any evidence of amelioration of the ischaemic damage to the CNS (Lanier *et al.*, 1990).

The influence of excitatory amino acid antagonists on the delayed degeneration of hippocampal CA1 pyramidal neurons in the gerbil and the rat has been the subject of intense investigation and controversy. A clear understanding of the biological and technical bases for divergent results from different laboratories is beginning to emerge. The crucial determinant of whether NMDA antagonists will be effective in ischaemia (whether focal or global) appears to be the severity of the insult and its impact on energy state (see Siesjö & Bengtsson, 1989; Wieloch *et al.*, 1989). In many models of global ischaemia and in the ischaemic core of a focal insult, complete energy failure occurs and NMDA antagonists are not efficacious. In contrast, in the ischaemic penumbra (and possibly in global models where benefit is reported with NMDA antagonists), energy state is less markedly disturbed and NMDA antagonists are clearly efficacious (see Siesjö & Bengtsson, 1989; Wieloch *et al.*, 1989). The difference between partial and complete breakdown of energy production in a diffuse insult like global ischaemia is likely to be highly marginal and extremely sensitive to a number of subtle factors such as anaesthetics, nutritional state, gender, strain (for discussion see Meldrum, 1990). Furthermore, it is now generally accepted that the small differences in brain temperature during and after transient ischaemia dramatically modify the amount of delayed neuronal damage (see Busto *et al.*, 1987; Minanisawa *et al.*, 1990). Buchan & Pulsinelli (1990) produced overwhelming evidence that the ability of MK-801 to provide neuroprotection in the gerbil was inextricably linked to hypothermia. Irrespective of how meticulously temperature is controlled (see Gill & Woodruff, 1990) there will always be concern that drug efficacy is due to hypothermia during the chronic survival period in global

ischaemia models (Buchan & Pulsinelli, 1990).

Although NMDA antagonists are not effective in preventing delayed neuronal death after *severe* global ischaemia, NBQX which blocks non-NMDA glutamate receptors has recently been shown to markedly reduce damage to the hippocampus and other brain areas in these severe models (Buchan *et al.*, 1991; Nellgård & Wieloch, 1992).

NMDA receptor antagonists as clinically useful drugs

Excitatory amino acid antagonists are among a wide range of compounds presently being developed as neuro-protective agents. There is an enormous list of drugs and lead compounds at various stages of preclinical or clinical development, e.g. aminosteroids, free radical scavengers, various ion channel blockers, kappa opiate agonists, naftidrofuryl, gangliosides, 5-hydroxytryptamine_{1A} (5HT_{1A}) agonists, 5HT₂-receptor antagonists, α_2 -adrenoceptor antagonists, cyclo-oxygenase and lipo-oxygenase inhibitors and others (Ginsberg & Scheinberg, 1991).

It is worth emphasising that among the different pharmacological classes of anti-ischaemic drugs, NMDA antagonists occupy a unique position; for no other class is there such a vast, consistent literature which documents anti-ischaemic efficacy. It is now generally accepted that NMDA blockade reduces brain damage in experimental focal ischaemia irrespective of,

- (a) the species used,
- (b) the experimental design (anaesthesia, chronic or acute survival, etc.)
- (c) the particular site within the NMDA receptor complex at which blockade is achieved,
- (d) or whether drug treatment is initiated before or in the first few hours after onset of ischaemia.

Anti-ischaemic efficacy is only one element in the selection of drugs for clinical evaluation. Safety and adverse effects are also of paramount importance in determining the utility of new drugs. It is already clear that the actions of NMDA antagonists (other than those relating to their ability to reduce brain damage) will influence the selection of the clinical target and the design of the clinical trials.

Competitive and non-competitive NMDA antagonists (MK-801 and CPP) depress respiration and induce hypercapnia (Kurumaji *et al.*, 1989). MK-801 increases blood pressure in conscious rats and chloralose-anaesthetised cats (Kurumaji *et al.*, 1989; Ozyurt *et al.*, 1988) but markedly decreases blood pressure in halothane anaesthetised rats (Bielenberg & Beck, 1991; Park *et al.*, 1988a); at high doses, D-CCPene induces hypotension in chloralose-anaesthetised cats (Bullock *et al.*, 1990b; Chen *et al.*, 1991). While these effects present minimal difficulties in some conditions (e.g. head injury patients already on a ventilator in an intensive care unit), in others (e.g. elderly stroke patients with other cardiovascular complications), they may restrict their use.

The administration of NMDA receptors antagonists alters the behaviour of all experimental animals studied hitherto including non-human primates (France *et al.*, 1989; Koek *et al.*, 1988). In primates, the behavioural

effects of NMDA antagonists include disruption of learning and memory, ataxia, sedation and ultimately anaesthesia. The central issue for clinical trials is not whether the drugs induce behavioural changes but the concentration at which the behavioural changes are manifest relative to the therapeutic doses. With non-competitive antagonists typified by MK-801, behavioural alterations are apparent at concentrations similar to those required for anti-ischaemic efficacy. For competitive antagonists, behavioural alterations occur at concentrations three to ten times greater than those required for anti-ischaemic effects and there may be a wider separation for polyamine site antagonists such as ifenprodil (compare the data for the mouse of Koek & Colpaert (1990) and Gotti *et al.* (1990)).

Autoradiographic mapping of the functional consequences of NMDA receptor blockade supports and extends the view which has emerged from behavioural studies. Non-competitive NMDA antagonists and competitive NMDA antagonists, at doses broadly comparable in terms of anticonvulsant potency and anti-ischaemic efficacy, induce markedly dissimilar alterations in function-related glucose use in the CNS (Kurumaji *et al.*, 1989; Nehls *et al.*, 1988). Pronounced dose-related increases in glucose use were observed throughout the limbic system after non-competitive NMDA receptor antagonists with marked reduction in function-related glucose use widespread in neocortex. In contrast, the effects on glucose use of competitive NMDA receptor blockade or blockade of the glycine site are numerically small and anatomically circumscribed (Hargreaves *et al.*, 1991; Kurumaji *et al.*, 1989).

These alterations in function-related energy generation are particularly important as they appear to be predictive of the reversible morphological alterations which are observed in some brain areas after NMDA antagonists. In the rat posterior cingulate cortex, the acute administration of non-competitive NMDA antagonists, MK-801 phencyclidine and ketamine, effects a dose-dependent cellular swelling and vacuolisation, particularly in the multipolar and pyramidal medium to large sized neurones

in layers III and IV. The cellular swelling and vacuolisation subsided 12 h after drug administration, and by 24 h after dizocilpine administration, the histological appearance of the tissue was essentially normal (Olney *et al.*, 1989). These reversible changes in neuronal structure are noted with MK-801 at doses (ED_{50} approx. 0.2 mg kg^{-1}) similar to those at which anti-ischaemic effects, anticonvulsant effects and increased glucose use are seen. Similar neuronal swelling and vacuolisation are also observed with competitive NMDA antagonists when administered intracerebrally and after systemic administration, although doses that are greater than those required to reduce ischaemic damage are necessary (McCulloch & Iversen, 1991).

There are a number of features that should be emphasised in relation to CNS structural changes seen after NMDA receptor blockade. First, these changes are highly circumscribed in their anatomical distribution. Secondly, neither the neuronal swelling nor the increase in glucose use are seen in the posterior cingulate cortex after repeated dizocilpine treatment. Thirdly, the metabolic activation of components of the limbic system after MK-801 and the neuronal swelling and vacuolisation response can be completely prevented by light halothane anaesthesia or centrally acting anticholinergic drugs. Fourthly, the alterations in neuronal structure are completely and rapidly (24 h) reversible (McCulloch & Iversen, 1991). Finally, the risk/benefit ratio in the clinical conditions (stroke, head trauma) in which NMDA antagonists could be used need to be considered; the occurrence of neuronal swelling in a few areas of limbic forebrain, has to be balanced against the normal outcome in stroke and head trauma – at best, significant volumes of cerebral tissue are irreversibly damaged leading to lasting disability or, at worst, the death of the patient. The use of these agents in patients at risk of brain damage is underpinned by the absence of doubt from preclinical investigations that NMDA receptor antagonists will prevent damage occurring to brain tissue in such clinical conditions if administered within a therapeutically relevant time window.

References

- Aebischer, B., Frey, P., Haerter, H.-P., Herrling, P. L. & Muller, W. (1989). Synthesis and NMDA antagonist properties of the enantiomers of 4-(3-phosphonopropyl)-piperazine-2-carboxylic Acid (CPP) and of the unsaturated analogue (E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPP-ene). *Helvetica Chimica Acta*, **72**, 1043–1051.
- Andiné, P., Lehmann, A., Ellren, K. *et al.* (1988). The excitatory amino acid antagonists kynurenic acid administered after hypoxia-ischemia in neonatal rats offers neuroprotection. *Neurosci. Lett.*, **90**, 208–212.
- Bielenberg, G. W. & Beck, T. (1991). The effects of dizocilpine (MK-801), phencyclidine and nimodipine on infarct size 48 h after middle cerebral artery occlusion in the rat. *Brain Res.*, **552**, 338–342.
- Buchan, A. & Pulsinelli, W. A. (1990). Hypothermia but not the N-methyl-D-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. *J. Neurosci.*, **10**, 311–316.
- Buchan, A. M., Li, H., Cho, S. & Pulsinelli, W. A. (1991). Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transient forebrain ischemia in adult rats. *Neurosci. Lett.*, **132**, 255–258.
- Bullock, R., Butcher, S. P., Chen, M.-H., Kendall, L. & McCulloch, J. (1991). Correlation of the extracellular concentration with extent of blood flow reduction after subdural hematoma in the rat. *J. Neurosurg.*, **74**, 794–802.
- Bullock, R., Graham, D. I., Chen, M.-H., Lowe, D. & McCulloch, J. (1990). Focal cerebral ischemia in the cat: pretreatment with a competitive NMDA receptor antagonist, D-CPP-ene. *J. Cereb. Blood Flow Metab.*, **10**, 668–674.
- Busto, R., Dietrich, W. D., Globus, M. Y.-T., Valdés, I., Scheinberg, P. & Ginsberg, M. D. (1987). Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J. Cereb. Blood Flow Metab.*, **7**, 729–738.
- Butcher, S. P., Bullock, R., Graham, D. I. & McCulloch, J. (1990). Correlation between amino acid release and neuropathologic outcome in rat brain following middle cerebral artery occlusion. *Stroke*, **21**, 1727–1733.

- Carter, C., Rivy, J. P. & Scatton, B. (1989). Ifenprodil and SL 820715 are antagonists at the polyamine site of the N-methyl-D-aspartate (NMDA) receptor. *Eur. J. Pharmacol.*, **164**, 611–612.
- Chen, M., Bullock, R., Graham, D. I., Frey, P., Lowe, E. & McCulloch, J. (1991). Evaluation of a competitive NMDA antagonist (D-CPPene) in feline focal cerebral ischaemia. *Ann. Neurol.*, **30**, 62–70.
- Choi, D. W., Koh, J.-Y. & Peters, S. (1988). Pharmacology of glutamate neurotoxicity in cortical cell culture: Attenuation by NMDA antagonists. *J. Neurosci.*, **8**, 185–196.
- Choi, D. W. (1991). Excitotoxicity. In *Excitatory amino acid antagonists*, ed. Meldrum, B. S., pp 216–236. Oxford: Blackwell.
- Dawson, V. L., Dawson, T. M., London, E. D., Bredt, D. S. & Snyder, S. H. (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl. Acad. Sci. USA*, **88**, 6368–6371.
- Dirnagl, U., Tanabe, J. & Pulsinelli, W. (1990). Pre- and posttreatment with MK-801 but not pretreatment alone reduces neocortical damage after focal cerebral ischemia in the rat. *Brain Res.*, **527**, 62–68.
- Fleischer, J. E., Tateishi, A., Drummond, J. C., Scheller, M. S., Grafe, M. R., Zornow, M. H., Shearman, G. T. & Shapiro, H. M. (1989). MK-801, an excitatory amino agonist, does not improve neurologic outcome following cardiac arrest in cats. *J. Cereb. Blood Flow Metab.*, **9**, 795–804.
- Foster, A. C. & Fagg, G. E. (1987). Taking apart NMDA receptors. *Nature*, **329**, 395–396.
- France, C. P., Woods, J. H. & Ornstein, P. (1989). The competitive N-methyl-D-aspartate (NMDA) antagonist CGS 19755 attenuates the rate-decreasing effects of NMDA in rhesus monkeys without producing ketamine-like discriminative stimulus effects. *Eur. J. Pharmacol.*, **159**, 133–139.
- Frandsen, A., Drejer, J. & Schousboe, A. (1989). Direct evidence that excitotoxicity in cultured neurons is mediated via N-methyl-D-aspartate (NMDA) as well as non-NMDA receptors. *J. Neurochem.*, **53**, 297–299.
- Gelmers, H. J., Gorter, K., De Weerd, C. J. & Wiezer, H. J. A. (1988). A controlled trial of nimodipine in acute ischemic stroke. *New Engl. J. Med.*, **318**, 203–207.
- George, C. P., Goldberg, M. P., Choi, D. W. & Steinberg, G. K. (1988). Dextromethorphan reduces neocortical ischemic neuronal damage *in vivo*. *Brain Res.*, **440**, 375–379.
- Gill, R., Brazell, C., Woodruff, G. N. & Kemp, J. A. (1991). The neuroprotective action of dizocilpine (MK-801) in the rat middle cerebral artery occlusion model of focal ischaemia. *Br. J. Pharmacol.*, **103**, 2030–2036.
- Gill, R., Foster, A. C. & Woodruff, G. N. (1987). Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *J. Neurosci.*, **7**, 3343–3349.
- Gill, R., Nordholm, L. & Lodge, D. (1992). The neuroprotective actions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline(NBQX) in a rat focal ischaemia model. *Brain Res.*, (in press).
- Gill, R. & Woodruff, G. N. (1990). The neuroprotective actions of kynurenic acid and MK-801 in gerbils are synergistic and not related to hypothermia. *Eur. J. Pharmacol.*, **176**, 143–149.
- Ginsberg, M. D. & Scheinberg, P. (eds) (1991). XVth International Symposium on Cerebral Blood Flow and Metabolism. *J. Cereb. Blood Flow Metab.*, **11**, Suppl. 2.
- Gotti, B., Benavides, J., MacKenzie, E. T. & Scatton, B. (1990). The pharmacotherapy of focal cortical ischaemia in the mouse. *Brain Res.*, **522**, 290–307.
- Gotti, B., Duverger, D., Bertin, J., Carter, C., Dupont, R., Frost, J., Gaudilliere, B., MacKenzie, E. T., Rousseau, J., Scatton, B. & Wick, A. (1988). Ifenprodil and SL 82.0715 as cerebral anti-ischemic agents. I. evidence for efficacy in models of focal cerebral ischemia. *J. Pharmacol. exp. Ther.*, **247**, 1211–1221.
- Hargreaves, R. J., Rigby, M., Smith, D., Foster, A., Hurley, C. J. & Gill, R. G. (1991). L-687,414(+) CIS-4-methyl-HA-966, an NMDA receptor antagonist acting at the glycine site, does not alter glucose metabolism or neuronal morphology at neuroprotective dose levels. *J. Cereb. Blood Flow Metab.*, **11**, S301.
- Hattori, H., Morin, A. M., Schwartz, P. J., Fujikawa, D. G. & Wasterlain, C. G. (1989). Posthypoxic treatment with MK-801 reduces hypoxic-ischemic damage in the neonatal rat. *Neurology*, **39**, 713–718.
- Hossmann, K.-A. (1982). Treatment of experimental cerebral ischemia. *J. Cereb. Blood Flow Metab.*, **2**, 275–297.
- Izumi, Y., Roussel, S., Pinard, E. & Seylaz, J. (1991). Reduction of infarct volume by magnesium after middle cerebral artery occlusion in rats. *J. Cereb. Blood Flow Metab.*, **11**, 1025–1030.
- Jones, T. J., Morawetz, R. B., Crowell, R. M., Marcoux, F. W., Fitzgibbon, S. J., Degirolami, U. & Ojemann, R. G. (1981). Thresholds of focal cerebral ischemia in awake monkeys. *J. Neurosurg.*, **54**, 773–782.
- Kemp, J. A., Foster, A. C., Leeson, P. D., Priestly, T., Tridgett, R. & Iversen, L. L. (1988). 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. USA*, **85**, 6547–6550.
- Kemp, J. A., Foster, A. C. & Wong, E. H. F. (1987). Non-competitive antagonists of excitatory amino acid receptors. *Trends neurol. Sci.*, **10**, 294–298.
- Kochhar, A., Zivin, J. A., Lyden, P. D. & Mazzarella, V. (1988). Glutamate antagonist therapy reduces neurologic deficits produced by focal central nervous system ischemia. *Arch. Neurol.*, **45**, 148–153.
- Koek, W., Woods, J. H. & Winger, G. D. (1988). MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. *J. Pharmacol. exp. Ther.*, **245**, 969–974.
- Koek, W. & Colpaert, F. C. (1990). Selective blockade of N-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: relationship with phencyclidine-like behavioral effects. *J. Pharmacol. exp. Ther.*, **252**, 349–357.
- Kurumaji, A., Nehls, D. G., Park, C. K. & McCulloch, J. (1989). Effect of NMDA antagonists MK-801 and CPP, upon local cerebral glucose use. *Brain Res.*, **496**, 268–284.
- Lanier, W. L., Perkins, W. J., Karlsson, B. R., Milde, J. H., Scheithauer, B. W., Shearman, G. T. & Michenfelder, J. D. (1990). The effects of dizocilpine maleate (MK-801), an antagonist of the N-methyl-D-aspartate receptor, on neurologic recovery and histopathology following complete cerebral ischemia in primates. *J. Cereb. Blood Flow Metab.*, **10**, 252–261.
- Lehmann, J., Hutchison, A. J., McPherson, S. E., Mondadori, C., Schmutz, M., Sinton, C. M., Tsai, C., Murphy, D. E., Steel, D. J., Williams, M., Cheyney, D. L. & Wood, P. L. (1988). DGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. *J. Pharmacol. exp. Ther.*, **246**, 65–76.
- Lodge, D. & Collingridge, G. (1990). Les agents provocateurs: a series on the pharmacology of excitatory amino acids. *Trends pharmacol. Sci.*, **11**, 22–23.
- Lucas, D. R. & Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Arch. Ophthalmol.*, **58**, 193–201.
- McCulloch, J., Bullock, R. & Teasdale, G. M. (1991). Excitatory amino acid antagonists: opportunities for the

- treatment of ischaemic brain damage in man. In *Excitatory amino acid antagonists*, ed. Meldrum, B. S., pp. 287–325. Oxford: Blackwell.
- McCulloch, J. & Iversen, L. L. (1991). Autoradiographic assessment of the effects of N-methyl-D-aspartate (NMDA) receptor antagonists *in vivo*. *Neurochem. Res.*, **16**, 951–963.
- McDonald, J. W., Silverstein, F. S. & Johnston, M. V. (1987). MK-801 protects the neonatal brain from hypoxic-ischemic damage. *Eur. J. Pharmacol.*, **140**, 359–361.
- McDonald, J. W., Silverstein, F. S. & Johnston, M. V. (1989). Neuroprotective effects of MK-801, TCP, PCP and CPP against N-methyl-D-aspartate induced neurotoxicity in an *in vivo* perinatal rat model. *Brain Res.*, **490**, 33–40.
- Meldrum, B. (1990). Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. *Cereb. Brain Metab. Rev.*, **2**, 27–57.
- Michaels, R. L. & Rothman, S. M. (1990). Glutamate neurotoxicity *in vitro*: antagonist pharmacology and intracellular calcium concentrations. *J. Neurosci.*, **10**, 283–292.
- Michenfelder, J. D., Lanier, W., Scheithauer, B. W., Perkins, W. J., Shearman, G. T. & Milde, J. H. (1989). Evaluation of the glutamate antagonist dizocilpine maleate (MK-801) on neurologic outcome in a canine model of complete cerebral ischemia: correlation with hippocampal histopathology. *Brain Res.*, **481**, 228–234.
- Minanishawa, H., Nordström, C.-H., Smith, M.-L. & Siesjö, B. K. (1990). The influence of mild body and brain hypothermia in ischaemic brain damage. *J. Cereb. Blood Flow Metab.*, **10**, 365–374.
- Nehls, D. G., Kurumaji, A., Park, C. K. & McCulloch, J. (1988). Differential effects of competitive and non-competitive N-methyl-D-aspartate antagonists on glucose use in the limbic system. *Neurosci. Lett.*, **91**, 204–210.
- Nellgård, B. and Wieloch, T. (1992). Postischaemic blockade of AMPA but not NMDA receptors mitigates neuronal damage in the rat brain following transient severe cerebral ischemia. *J. Cereb. Blood Flow Metab.*, **12**, 2–11.
- Nicholls, D. & Attwell, D. (1990). The release and uptake of excitatory amino acids. *Trends pharmac. Sci.*, **11**, 462–468.
- Olney, J. W., Labruyere, J. & Price, M. T. (1989). Pathological changes induced in cerebrocortical neurons by phenylcyclidine and related drugs. *Science*, **244**, 1360–1362.
- Osborne, K. A., Shigeno, T., Balarsky, A. M., Ford, I., McCulloch, J., Teasdale, G. M. & Graham, D. I. (1987). Quantitative assessment of early brain damage in a rat model of focal cerebral ischaemia. *J. Neurol. Neurosurg. Psychiatr.*, **50**, 402–410.
- Ozyurt, E., Graham, D. I., Woodruff, G. N. & McCulloch, J. (1988). Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischemia in the cat. *J. Cereb. Blood Flow Metab.*, **8**, 138–143.
- Park, C. K., McCulloch, J., Kang, J. K. and Choi, C. R. (1991). Comparison of competitive and non-competitive NMDA antagonists in focal cerebral ischaemia in rats. *J. Cereb. Blood Flow Metab.*, **11**, S299.
- Park, C. K., Nehls, D. G., Graham, D. I., Teasdale, G. M. & McCulloch, J. (1988a). The glutamate antagonist MK-801 reduces focal ischaemic brain damage in the rat. *Ann. Neurol.*, **24**, 543–551.
- Park, C. K., Nehls, D. G., Graham, D. I., Teasdale, G. M. & McCulloch, J. (1988b). Focal cerebral ischaemia in the cat: treatment with the glutamate antagonist MK-801 after induction of ischaemia. *J. Cereb. Blood Flow Metab.*, **8**, 757–762.
- Pickard, J. D., Murray, G. D., Illingworth, R., Shaw, M. D. M., Teasdale, D. M., Foy, P. M., Humphrey, P. R. D., Lang, D. A., Nelson, R., Richards, R., Sinar, J., Bailey, S. & Skene, A. (1989). Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. *Br. med. J.*, **298**, 636–642.
- Prince, D. A., & Feeser, H. R. (1988). Dextromethorphan protects against cerebral infarction in a rat model of hypoxia-ischaemia. *Neurosci. Lett.*, **85**, 291–296.
- Rothman, S. M. & Olney, J. W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann. Neurol.*, **19**, 105–111.
- Roussel, S., Pinard, E. & Seylaz, J. (1990). Kynurenate does not reduce infarct size after middle cerebral artery occlusion in spontaneously hypertensive rats. *Brain Res.*, **518**, 353–355.
- Roussel, S., Pinard, E. & Seylaz, J. (1992). Effect of MK-801 on focal brain infarction in normotensive and hypertensive rats. *J. Hypertension*, **19**, 40–46.
- Shimada, N., Graf, R., Rosner, G., Wakayama, A., George, C. P. & Heiss, W.-D. (1989). Ischemic flow threshold for extracellular glutamate increase in cat cortex. *J. Cereb. Blood Flow Metab.*, **9**, 603–606.
- Siesjö, B. K. & Bengtsson, F. (1989). Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: A unifying hypothesis. *J. Cereb. Blood Flow Metab.*, **9**, 127–140.
- Simon, R. & Shiraishi, K. (1990). N-methyl-D-aspartate antagonist reduces stroke size and regional glucose metabolism. *Ann. Neurol.*, **27**, 606–611.
- Singh, L., Donald, A. E., Foster, A. C., Hutson, P. H., Iversen, L. L., Iversen, S. D., Kemp, J. A., Leeson, P. D., Marshall, G. R., Oles, R. J., Priestly, T., Thorn, L., Tricklebank, M. D., Vass, C. A. & Williams, B. J. (1990). Enantiomers of HA-966 (3-amino-1-hydroxypyrrolid-2-one) exhibit distinct central nervous system effects: (+)-HA-966 is a selective glycine/N-methyl-D-aspartate receptor antagonist, but (–)-HA-966 is a potent γ -butyrolactone-like sedative. *Proc. Natl. Acad. Sci. USA.*, **87**, 347–351.
- Steinberg, G. K., George, C. P., DeLaPaz, R., Shibata, D. K. & Gross, T. (1988). Dextromethorphan protects against cerebral injury following transient focal ischemia in rabbits. *Stroke*, **19**, 1112–1118.
- Steinberg, G. K., Kunis, D., Saleh, J. & DeLaPaz, R. (1991). Protection after transient focal cerebral ischemia by the N-methyl-D-aspartate antagonists dextrorphan is dependent upon plasma and brain levels. *J. Cereb. Blood Flow Metab.*, **11**, 1015–1024.
- Sterz, F., Leonov, Y., Safer, P., Radovsky, A., Stezoski, W., Reich, H., Sherman, G. T. & Greber, T. F. (1989). Effect of excitatory amino acid receptor blocker MK-801 on overall neurologic, and morphologic outcome after prolonged cardiac arrest in dogs. *Anesthesiol.*, **71**, 907–918.
- Uematsu, D., Araki, N., Greenberg, J. H., Sladky, J. & Reivich, M. (1991). Combined therapy with MK-801 and nimodipine for protection of ischemic brain damage. *Neurology*, **41**, 88–94.
- Wallace, M. C., Teasdale, G. M. & McCulloch, J. (1992). Autoradiographic analysis of ^3H -Dizocilpine (MK-801) *in vivo* uptake and *in vitro* binding after focal cerebral ischemia in the rat. *J. Neurosurg.*, **76**, 127–133.
- Wieloch, T., Gustafson, I. & Westerberg, E. (1989). The NMDA antagonist, MK-801, is cerebroprotective in situations where some energy production prevails but not under conditions of complete energy deprivation. *J. Cereb. Blood Flow Metab.*, **9**, S6.
- Wong, E. H. F., Kemp, J. A., Priestly, T., Knight, A. R., Woodruff, G. N. & Iversen, L. L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. USA.*, **83**, 7104–7108.
- Zambramski, J. M., Spetzler, R. F. & Lee, K. S. (1991). Postischemic treatment with the NMDA-receptor antagonist MK-801 reduces cerebral injury after temporary focal ischemia in a primate model. *J. Cereb. Blood Flow Metab.*, **11**, S290.