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Pharmacological Approaches to the Treatment of Ischaemic Neuronal Damage

L. L. IVERSEN
Harlow

Summary
Retina is particularly susceptible to ischaemic damage following exposure to anoxia or hypoglycaemia. In animal models the neuronal damage following ischaemia resembles that caused by exposure to glutamate or other excitotoxic agents which act on excitatory amino acid receptors. There are a number of pharmacological approaches designed to limit neuronal damage following ischaemia. These include free radical scavenging agents, calcium channel blockers, kappa opiate agonists and excitatory amino acid antagonists. Recent studies with antagonists acting at both NMDA and non-NMDA receptors for glutamate show that such compounds can protect against ischaemic damage, and are effective even when administered several hours after the ischaemic insult.

Retinal neurones, like those in other regions of CNS, are highly dependent on the oxidative metabolism of glucose for their energy requirements, and are susceptible to damage under conditions of ischaemia or hypoglycaemia. There has been an increased research effort in recent years devoted to the development of pharmacological agents which might protect neural tissue against such damage. A wide variety of different scientific approaches has been used, and new compounds in development as potential 'neuroprotectives' include amino steroids and other free radical scavengers, calcium channel blockers, kappa opiate agonists, and excitatory amino acid antagonists (for reviews see 1–3). This short review will focus on the latter category of new drugs, where considerable scientific progress has been made recently.

The Excitoxic Hypothesis

The neurotoxic effects of L-glutamate and L-aspartate were first described by Lucas and Newhouse[4] 33 years ago. They found that systemic administration of L-glutamate or aspartate in very large doses (2–4 g/kg s.cut) lead to profound neurodegenerative changes in retinal neurones of albino mice. These changes were seen mainly in ganglion cells in adult animals, but in immature animals all retinal cells were affected. Olney and colleagues more than a decade later noted that systemically administered L-glutamate caused neurodegenerative changes in basal hypothalamus and circumventricular regions of immature rat brain, and Olney was the first to formulate the hypothesis that L-glutamate and other neurotoxins which act to excite nerve cells owe their toxicity to their excitant properties.[5,6] It is now generally recognised that L-glutamate and L-aspartate are widely used excitatory neurotransmitters in mammalian CNS.[6,7] In retina they are the principal transmitters used by excitatory inter-neurones (bipolar cells, some amacrine cells and probably most ganglion cells).

A further extension of the excitotoxin hypothesis suggests that neural damage fol-

Correspondence to: L. L. Iversen, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK.

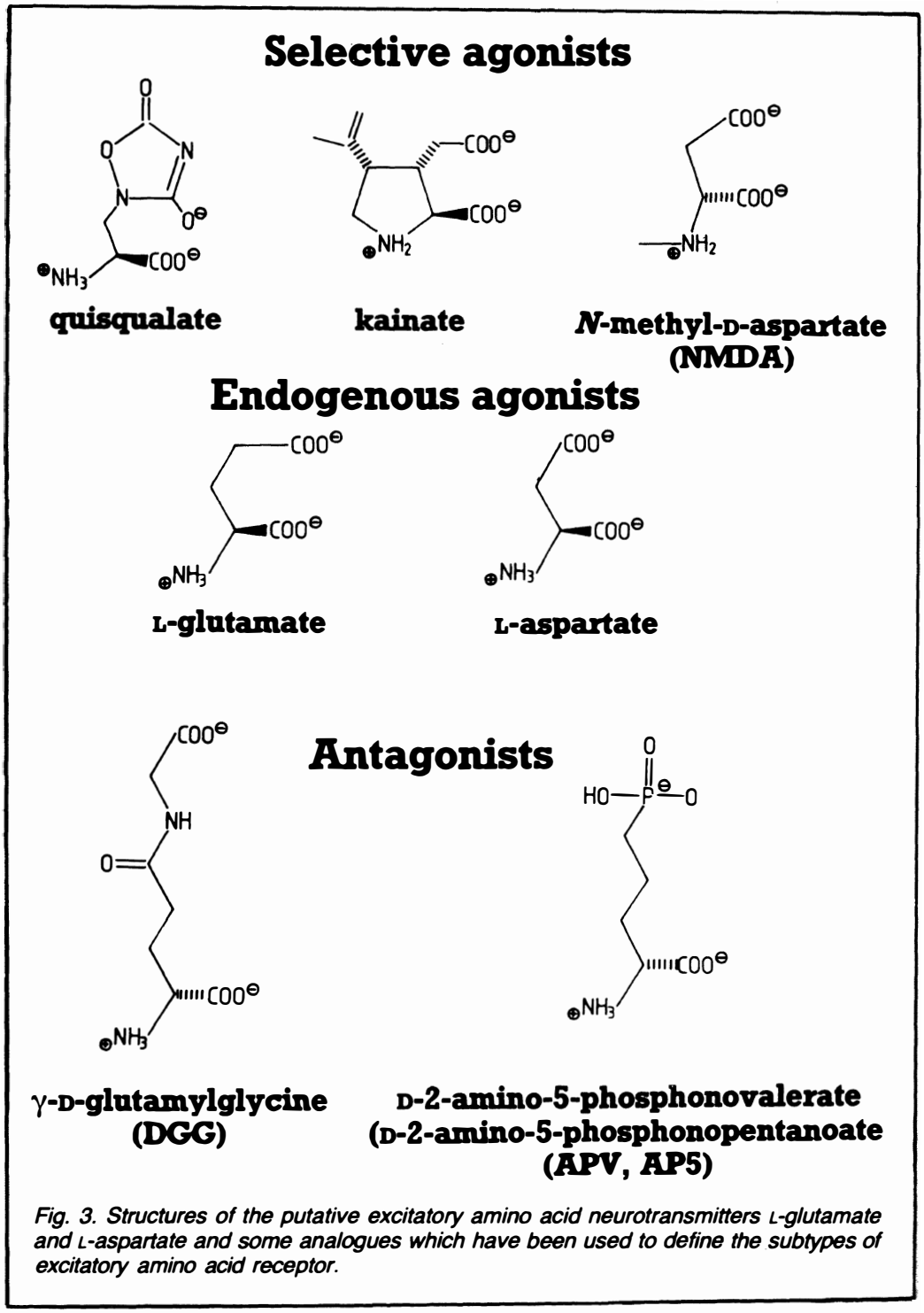

Fig. 3. Structures of the putative excitatory amino acid neurotransmitters L-glutamate and L-aspartate and some analogues which have been used to define the subtypes of excitatory amino acid receptor.

Fig. 1. *Structures of excitatory amino acid neurotransmitters and some analogues which have been used to define the receptor sub-types.*

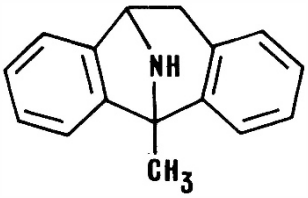

Fig. 2. *DIZOCILPINE (MK-801) = (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine.*

lowing ischaemia or hypoglycaemia may result partly as a consequence of increased release of glutamate and/or over stimulation of excitatory amino acid receptors during such conditions.[8,9]

Excitatory Amino Acid Antagonists as Neuroprotective Agents

L-glutamate and related excitants act on several different receptors in CNS. At least three major receptor sub-types are recognised: the N-methyl-D-asparate preferring (NMDA) receptor, the quisqualate/kainate receptor (activated selectively by these agonists) and a 'metabotropic' quisqualate preferring receptor, in which recognition of agonist is coupled to breakdown of membrane phosphoinositides. The first two receptors control cation channels, allowing the entry of sodium or sodium and calcium (NMDA) to depolarise target neurones (Fig. 1).

A range of anatogonists acting at the NMDA receptor have been described; these include competitive antagonists of the amino phosphonate series, and non-competitive antagonists. The most potent of the latter is the research compound MK-801 (dizocilpine) (Fig. 2), others include the intravenous anaesthetics ketamine and phencyclidine, and the

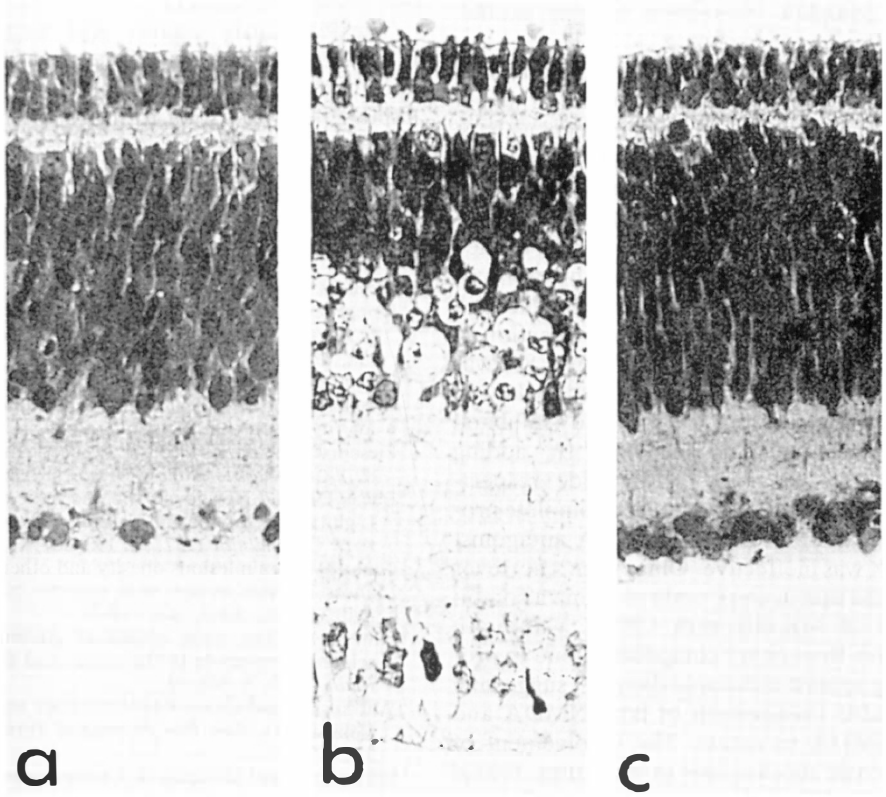

Fig. 3. *Light micrograph of 15 day old chick embryo retina following 30 min incubation in balnced salt solution containing no excitotoxin (a), or 200 μM NMDA (b), or 200 μM NMDA + 0.1 μM MK-801 (c). The typical pattern of neuronal necrosis induced by NMDA is apparent in (b), including marked thickening of inner neural layers due to oedema. Retina in (c) although exposed to the same excitotoxin was completely protected by MK-801. From Olney et al.* (ref. 14).

opiate derivatives dextromethorphan.[10–12] There has been less progress in identifying antagonists acting on non-NMDA receptors, although recently quinoxaline derivatives have been discovered which act in this way, eg CNQX.[13]

NMDA antagonists, both of the competitive and non-competitive types have been found to offer protection against the neural damage normally evoked by L-glutamate, NMDA and other excitoxins which act at NMDA receptors. Such experiments have been performed using neurones in tissue culture or in isolated fragments of CNS tissue *in vitro,* and also *in vivo*. For example, Olney *et al.*[14] found that MK-801 was remarkably effective in preventing the histological damage normally evoked by exposure of 15 day old chick retina to 200 μM NMDA *in vitro*. A concentration of 0.1 μM MK-801 wa able to offer complete protection in this model. (Fig. 3).

Other experiments have attempted to simulate conditions of ischaemia and/or hypoglycaemia *in vitro*. Choi and colleagues have used primary cultures of mouse cortical neurones for such studies, and exposed them to a brief period of hypoxia (4–6h) or to glucose-free medium. The damage which ensued appeared to be mediated largely by excitoxic mechanisms, since MK-801 and other NMDA antagonists provided complete protection in this *in vitro* model. The EC50 for MK-801 was 0.1 μM.[15] Zeevalk and Nicklas used 13 day old chick embyro retina *in vitro* and simulated hypoglycaemia and ischaemia by adding iodoacetate or potassium cyanide respectively.[16] Again MK-801 afforded complete protection, whereas the non-NMDA antagonist CNQX was ineffective. Olney *et al.*[17] however reported that in chick embryo retina a combination of MK-801 with CNQX was more effective than either compound alone in protecting against ischaemic damage, suggesting a possible involvement of both NMDA and non-NMDA receptors. The involvement of excitotoxic mechanisms in mediating retinal damage after ischaemia was strongly suggested by the findings of David *et al.*[18] who found that damage elicited by exposure of chick embryo retina to a 40 minute period of hypoxia could be largely prevented by addition of the non-selective glutamate antagonist gamma-glutamylglycine. Levy and Lipton[19] have described an *in vitro* model using rat retinal ganglion cells in culture, and found that the damage elicited by exposure to elevated external calcium concentrations (10 mM) was prevented by addition of NMDA antagonists, suggesting mediation by glutamate. They found that addition of MK-801 could be delayed for 4 h after initial exposure to elevated calcium, and still remain neuroprotective. Other studies using a variety of *in vivo* models of cerebral ischaemia or exitotoxin-induced damage to hippocampal or cortical neurones have also revealed a delayed efficacy of MK-801 in offering neuroprotection, suggesting a possible clinical utility of this or related NMDA antagonists in the acute treatment of stroke, prenatal-ischemia or other forms of cerebral ischaemia or hypoglycaemia (for reviews see 20–21).

The excitatory amino acid antagonists represent a promising new approach to the development of neuroprotective agents, although it is too soon to judge whether the effectiveness of such compounds in laboratory models will translate into clinical practice.

Key words: ischaemic, glutamate, NMDA receptor, neuroprotective agents, MK-801, dizocilpine.

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