ELSEVIER

European Journal of Pharmacology 276 (1995) 55–60

ejp

Acute effects of irradiation on the rat brain: protection by glutamate blockade

Faouzia Alaoui [a], Jeremy Pratt [b,*], Suzanne Trocherie [a], Louis Court [a], Jean-Marie Stutzmann [b]

[a] *Laboratoire de Neurobiologie du Groupe de Radiobiologie du Service de Santé des Armées, CEA / DSV / DPTE / SSA, BP 6, 92265 Fontenay aux Roses Cedex, France*

[b] *Service 'Maladies Neurodégénératives', Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 94403 Vitry Cedex, France*

Received 6 October 1994; revised 8 December 1994; accepted 30 December 1994

Abstract

Riluzole (2-amino-6-trifluorothethoxy benzothiazole), dizocilpine (MK-801; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)-cyclohepten-5,10-imine maleate), and lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2-triazine), agents reported to have neuroprotective actions, and WR2721 (*S*-2-(3-aminopropylamino)-ethylphosphorothioic acid), a radioprotector, were evaluated in 15-day-old rats that underwent a 2.5 Gray (Gy) irradiation from a cobalt 60 source. 20 min after irradiation, riluzole (0.5–8 mg/kg), dizocilpine (0.1–1 mg/kg), lamotrigine (25 mg/kg), WR 2721 (75 mg/kg) or vehicle, were injected intraperitoneally. 6 h after irradiation, behavioural and histological evaluations revealed that exposure to 2.5 Gy caused hypolocomotion, stumbling gait and somnolence, which was significantly reduced, from the dose of 4 mg/kg i.p. of riluzole. A dose-dependent protection of neurones in the dentate gyrus, starting from the dose of 1 mg/kg i.p. was also seen. Dizocilpine caused behavioural modifications but significantly reduced neuronal damage. Lamotrigine significantly increased neuronal damage while WR 2721 conferred no protection. In conclusion, two blockers of glutamatergic neurotransmission conferred significant protection against brain damage caused by ionizing irradiation when administered subsequent to exposure.

Keywords: Ionizing irradiation; Neuroprotective; Glutamate neurotransmission; Na^+ channel blocker; Neuronal damage; (Rat)

1. Introduction

The mammalian brain is damaged by ionizing irradiation. Such exposure may occur during industrial or military accidents or as a part of therapy for cancer. Irradiation remains one of the major treatments for certain types of cancer and such therapy may necessitate cephalic or whole body irradiation. Relatively low doses of gamma, X or neutron irradiation of mammals, including humans, induce numerous functional disorders and provoke a transient encephalopathy depending on absorbed dose, distribution and dose rate. Irradiation between 2.5 and 4.5 Gray (Gy), which is of the same order as routinely used in clinical radiotherapy, when given to rabbits causes significant disturbance of the electrical activity of hippocampal pyramidal neurones (Bassant and Court, 1978). Simultaneously, large localized lesions are seen in the dentate gyrus and pycnotic cells are found at the base of the granular cell layer of the dentate gyrus and in the sub-granular zone less than 24 h after irradiation and are cleared up by astrocytes and microglia responsible for the phagocytosis of dead cells (Gueneau et al., 1982). Several proliferative zones subsist in the brain of immature rats and rabbits including the subgranular zone of the dentate gyrus, the external granular layer of the cerebellum, and the subependymal layer of the olfactory bulb (Altman and Das, 1965; Gueneau et al., 1982) and these zones are very sensitive to radiation. The development of the hippocampal dentate gyrus provides an interesting experimental model with which to study radiation-induced lesions and examine the value of a post-exposure drug treatment. The post-natal neurogenesis that occurs in the granular and sub-granular layers of the dentate gyrus is substantial and continues

* Corresponding author. Tel. (33) 1 45738892, fax (33) 1 45737653.

SSDI 0014-2999(95)00006-2

up to 21 days after birth (Bayer and Altman, 1974). The object of this study was to evaluate the effect of four neuroprotective compounds, riluzole (2-amino-6-trifluorothethoxy benzothiazole), dizocilpine (MK-801; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine maleate), lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2-triazine), and WR 2721 (*S*-2-(3-aminopro-pylamino)-ethylphosphorothioic acid), on behavioural and histological criteria in immature rats, when compounds were administered after irradiation. Riluzole, dizocilpine and lamotrigine have all shown interesting protective effects in models of cerebral ischaemia, when administered after the insult (Park et al., 1988; Pratt et al., 1992; Wahl et al., 1993; Leach et al., 1991; Rataud et al., 1994) while WR 2721, a phosphorothioate radioprotector, has been shown to protect many organs, including the rat spinal cord, after intrathecal administration, from the effects of ionizing irradiation (Spence et al., 1988). Dizocilpine acts via the inhibition of the *N*-methyl-D-aspartate (NMDA) glutamate channel (Wong et al., 1986), while lamotrigine is a Na^+ channel blocker (Leach et al., 1986) with inhibition of glutamate release as a secondary effect (Leach et al., 1991). Riluzole has activity both on flux through the NMDA channel, though without showing any affinity for glutamate binding sites (Debono et al., 1993), and on the voltage-dependent Na^+ channel (Benoit and Escande, 1991; Hebert et al., 1994). WR 2721 is thought to act via modification of available oxygen (Durand and Olive, 1989).

2. Materials and methods

Male Sprague-Dawley (IFFA-CREDO, France) rats, 15 days of age, weighing 28–35 g and maintained with their mothers, were used. A cobalt 60 source of gamma irradiation at the Centre d'Etudes Nucléaires de Fontenay aux Roses gave an exposure rate of 0.19 Gy/min. The rats were placed in an aerated plexiglass box which was rotated by 360° so animals would be irradiated evenly from all directions with a single dose of 2.5 Gy. Locomotor activity was observed 6 h post-irradiation. The rats were placed in a rectangular open field of 70 cm × 90 cm, divided into four equally sized rectangles, which contained sawdust from their home cage and in which their mother had been placed for 5 min immediately prior to the test. Squares crossed and rearing actions were noted over a 3-min period, as was a qualitative assessment of the animal's behaviour with any incidence of somnolence being noted as well as any stumbling or paralysis of the hind quarters. The observer was unaware of the treatment each animal had received. For histological studies similarly prepared animals were anaesthetized with sodium pentobarbital 6 h after irradiation and prepared for intra-aortic perfusion. A pretreatment with heparin (250 IU) to avoid coagulation as well as 0.3 ml of 1% sodium nitrite, to dilate the vessels and aid removal of red blood cells was perfused into the left ventricle. This was followed by a liquid fixative consisting of 1% paraformaldehyde, 1% glutaraldehyde and 0.05% $CaCl_2$ in a 0.1 M phosphate buffer at pH 7.3. Gravity ensured the flow of the perfusion liquid. After the perfusion, the animal's head was cut off. The brain was removed and immersed in liquid fixative and then left overnight at 4°C. The day after the perfusion, the frontal portion of the dentate gyrus was excised under a dissecting microscope. The fragments were immersed for 5 min in a wash solution, dehydrated in baths containing increasing concentrations of alcohol, then embedded in araldite. Serial sections 1 μm in width were made with an Ultra microtome (Reichert). They were stained over heat with a filtered solution of 1% toluidine blue (60°C) prepared in 1% borate buffer, then observed under an Orthoplan microscope. Three non-serial sections separated by 10 μm were examined for each animal by a histologist blinded as to the treatment protocol. The number of pycnotic cells per 1000 cells in the granular and subgranular layers was expressed as a proportion of surviving cells in the zone.

2.1. Drugs

Treatments were administered by intraperitoneal route 20 min after irradiation with control animals receiving vehicle alone. Riluzole (Rhône-Poulenc Rorer) was given at: 0.5, 1, 2, 4 and 8 mg/kg, (+)-dizocilpine (Research Biochemicals) was given at 0.1, 0.3 and 1 mg/kg, lamotrigine (Rhône-Poulenc Rorer) was administered at 25 mg/kg and WR 2721 (Professor Mijiniac, Hôpital de Poitiers, France) was given at 75 mg/kg. The results obtained for the control animals and the irradiated, treated animals were subjected to an analysis of variance for independent groups (Krushkall-Wallis test). Dunnett's test was used for establishing the significance of differences.

3. Results

A single dose of 2.5 Gy of ionizing irradiation caused a greatly increased evidence of somnolence and erratic gait (Fig. 1). Somnolence was noted in only 5 out of 35 sham-manipulated animals compared with 24 out of 30 irradiated animals ($P < 0.001$), while only 1 sham-treated animal showed stumbling against 21 of the 28 irradiated animals. A further two irradiated animals were left out of the assessment because they remained immobile and therefore could not be assessed. This sedation was quantified in the open field where a great fall in locomotor activity was evident following expo-

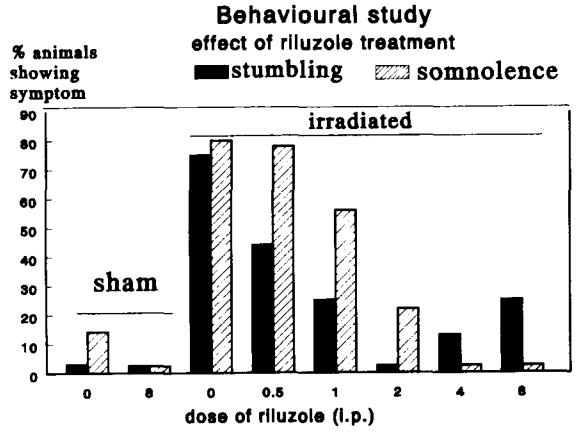

Fig. 1. Qualitative evaluation of animal behaviour carried out during passage through the open field, with the presence of a single period of somnolence or stumbling being counted as positive, while only complete absence of these signs was counted as negative. Sham: 0 ($n = 35$); sham-irradiated rats treated with riluzole at 8 mg/kg i.p. (sham: 8, $n = 11$); rats receiving 2.5 Gray of irradiation followed by vehicle treatment (0, $n = 30$) and animals irradiated with 2.5 Gray, then treated with 0.5 ($n = 9$), 1 ($n = 9$), 2 ($n = 8$), 4 ($n = 15$) or 8 ($n = 8$) mg/kg of riluzole i.p. Chi-square test for trend gives significant reductions in both parameters ($P < 0.001$) after riluzole treatment of irradiated animals.

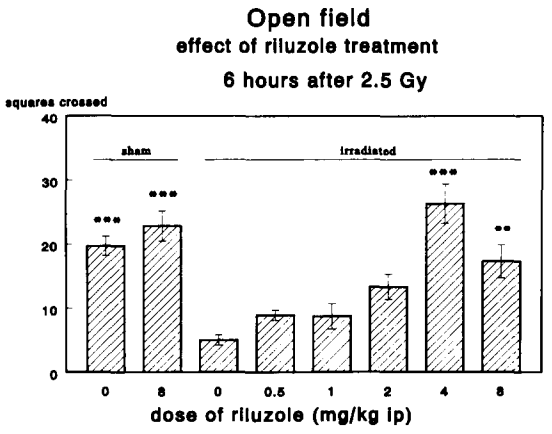

Fig. 2. The number of squares crossed in an open field over a period of 3 min for sham-treated and irradiated rats. Groups as per Fig. 1. The data are means ± S.E.M. $^{**}P < 0.01$, $^{***}P < 0.001$.

sure to irradiation (Fig. 2). Administration of a single dose of riluzole 20 min after the start of irradiation exposure reduced evidence of somnolence and stumbling, in both cases with a $P < 0.001$ in the chi-square test for a trend (Fig. 1). This again was supported by the open field test, where significant reversal of the hypolocomotion could be seen from the dose of 4 mg/kg (Fig. 2). Administration of riluzole at 8 mg/kg to sham-treated rats 6 h before testing did not significantly change behavioural responses from those seen for sham-treated animals which had received vehicle treatment alone and no stereotypic behaviour, wild running or profound sedation was noted. Treatment with 0.1 and 0.3 mg/kg of dizocilpine corrected the locomotor deficit ($P < 0.001$ versus irradiated controls) seen in the open field after irradiation (Fig. 3a). However stereotypic behaviour with head weaving, scratching, crying and stumbling was noted in both sham-treated and irradiated animals. At the highest dose of 1 mg/kg, dizocilpine caused profound sedation, again in both irradiated and sham-treated animals, preventing behavioural evaluation. Lamotrigine had no significant effects on behaviour of either the irradiated or sham-treated animals (Fig. 3a,b). WR-2721 was only examined in irradiated animals, where it did not reduce stumbling or somnolence, or modify motility, when compared to control irradiated animals (Fig. 3a). In histological studies exposure to a single dose of 2.5 Gy irradiation caused a dramatic increase in the number

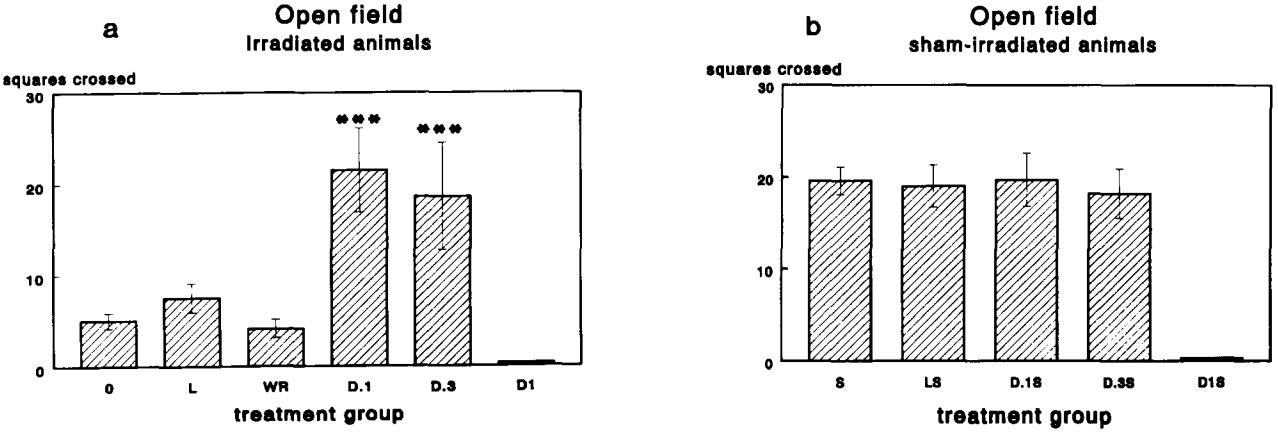

Fig. 3. (a) The number of squares crossed in an open field over the period of 3 min for irradiation followed by vehicle treatment (0, $n = 30$), irradiation followed by lamotrigine treatment (L, $n = 10$), irradiation followed by WR 2721 treatment (WR, $n = 10$), irradiation followed by dizocilpine at 0.1, 0.3 or 1 mg/kg (D.1, D.3 or D1 respectively). Treated animals compared with irradiated control group (0). $^{***}P < 0.001$. (b) Sham-irradiated control groups corresponding to treatment groups in (a). Sham, irradiated (S, $n = 35$), sham followed by lamotrigine treatment (LS, $n = 10$), or sham followed by dizocilpine (D.1S, D.3S or D1S).

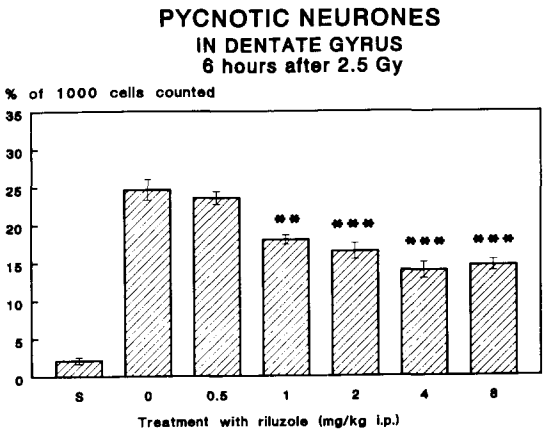

Fig. 4. Percentage of neurones in granular and sub-granular zones of the dentate gyrus showing pycnosis 6 h after treatment. S = sham irradiation followed by vehicle treatment, 0 = irradiation followed by vehicle treatment, 0.5, 1, 2, 4 and 8 = irradiation followed by treatment with riluzole at 0.5–8 mg/kg ($n = 12$/group, means ± S.E.M., $^{**}P < 0.01$, $^{***}P < 0.001$).

of pycnotic neurones in the dentate gyrus, reaching 25% of the neurones in this zone (Fig. 4). Treatment with riluzole provided significant protection of these neurones from the dose of 1 mg/kg and did not generate pycnotic cells in a group of sham-irradiated rats. Likewise treatment with dizocilpine conferred significant protection of neurones at both 0.3 and 1 mg/kg ($P < 0.01$; Fig. 5). Lamotrigine had the opposite effect, significantly increasing the number of pycnotic cells in the dentate gyrus, while WR-2721 had no protective effect in this model (Fig. 5).

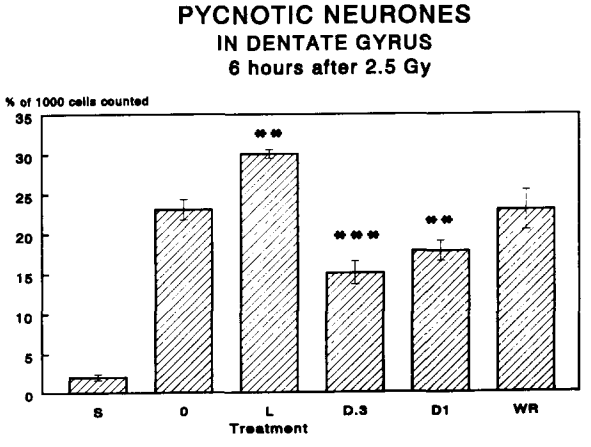

Fig. 5. Percentage of neurones in granular and sub-granular zones of the dentate gyrus showing pycnosis 6 h after treatment. S = sham irradiation followed by vehicle treatment, 0 = irradiation followed by vehicle treatment,L = irradiation followed by 25 mg/kg lamotrigine, D.3 = irradiation followed by dizocilpine at 0.3 mg/kg, D1 = irradiation followed by dizocilpine at 1 mg/kg, WR = irradiation followed by WR-2721 at 75 mg/kg ($n = 12$/group, means ± S.E.M., $^{**}P < 0.01$, $^{***}P < 0.001$).

4. Discussion

This study demonstrated that a single dose of 2.5 Gy of ionizing irradiation causes measurable behavioural deficits, and structural damage in the dentate gyrus of immature rats. This damage may be reduced by retrospective treatment with riluzole or dizocilpine. These two compounds have very different structures and biochemical properties but both block glutamate neurotransmission. Riluzole also shows a Na^+ channel inactivating property (Benoit and Escande, 1991), acting on the α sub-unit of the major Na^+ channel in the rat brain (Hebert et al., 1994). This compound is currently in clinical trial in amyotrophic lateral sclerosis as a neuroprotective agent (Bensimon et al., 1994). Riluzole's action on glutamate transmission has not been completely elucidated (see Stutzmann and Doble, 1994 for review) but it can be separated from the anti-Na^+ effect, and is sensitive to pertussis toxin, suggesting the role of a G-protein in the mediation of riluzole's action (Hubert et al., 1994). Riluzole non-competitively blocks currents carried by NMDA receptors expressed in *Xenopus* oocytes (Debono et al., 1993), shows potent anticonvulsant properties against glutamate- and aspartate-induced convulsions and is able to antagonize the stimulation of acetylcholine release by NMDA (Bénavidès et al., 1985; Mizoule et al., 1985). Riluzole also inhibits glutamate release both in vitro, in the CA1 area of the rat hippocampal slice (Martin et al., 1991), and in vivo in the anaesthetised cat (Chéramy et al., 1992). The compound however shows no affinity for any of the known binding sites of the glutamate receptors, unlike dizocilpine, which is a non-competitive blocker in the ion channel coupled to the NMDA receptor, possessing potent anti-convulsant properties (Wong et al., 1986). Both riluzole and dizocilpine have been shown to have neuroprotective effects in the same models of global and focal ischaemia at the doses used in the present study (Pratt et al., 1992; Gill and Kemp, 1989; Park et al., 1988). Lamotrigine is also active in neuroprotective models at doses similar to those used in this study (Graham et al., 1994; Rataud et al., 1994). It is a Na^+ channel blocker (Leach et al., 1986) currently in the clinic as an anti-epileptic agent. Lamotrigine inhibits veratridine-induced, though not K^+-induced, glutamate release (Leach et al., 1991), in contrast to riluzole (Martin et al., 1991). Lamotrigine may therefore reduce glutamate transmission, predominantly presynaptically, but the dose tested in this study conferred no protection against the radiation damage. This might suggest that intervention postsynaptically, as is the case of riluzole and dizocilpine, is necessary to prevent a glutamate component of acute radiation damage in this model, although a reduced sensitivity to lamotrigine in the 15 day post-natal rat cannot be ruled out after study of a single dose. Phos-

phorothioate radioprotectors such as WR-2721, have shown efficacy in a number of experimental models, and subsequently in the clinic. Unfortunately these compounds are highly water soluble and do not pass the blood-brain barrier, thus providing no protection for the brain (Yuhas and Storer, 1969). WR-2721 was included in this trial in the hope that, as the blood-brain barrier is disrupted by irradiation, the compound might show some efficacy. Its lack of effect might be due to lack of passage into the brain, or because modification of tissue oxygen metabolism is no longer relevant to the degree of radiation damage once the irradiation has ceased.

In the open field test, normal and sham-irradiated young rats showed considerable exploratory activity, presumably motivated by the odour of their mother and in an attempt to find a way through to her, while irradiation greatly reduced this behaviour. Treatment with riluzole or dizocilpine corrected behavioural parameters while only providing partial, though significant reductions in pycnotic neurones of the dentate gyrus. The difference between these two tests may be due to a threshold effect, whereby a certain degree of structural damage can be tolerated without impinging upon behaviour, or it may indicate that other factors are involved in the behavioural consequences of whole body irradiation and that riluzole and dizocilpine confer protection upon other structures.

Radiation damage is caused by generation of free radicals, mostly generated by the splitting of water molecules. These damage many components of the cell, including the DNA and lipid membranes, where oxygen-consuming chain reactions are started (Halliwell and Gutteridge, 1986). Free radicals may modify glutamate release and metabolism (Oliver et al., 1990; Pellegrini-Giampetro et al., 1990). Recently, using cerebellar granule cells in culture, Bockaert and colleagues have demonstrated the first direct relationship between O_2^- generation and excitatory amino acid-induced neuronal death since 5,5-dimethyl pyrroline 1-oxide, a spin trap, considerably reduced NMDA-induced neurotoxicity (Lafon-Cazal et al., 1993). The present study suggests that glutamate excitotoxicity may play some part in the acute neuronal damage subsequent to irradiation of the brain. There is no evidence in the literature of glutamate antagonists being used as radiorestorative agents, that is to say being given after irradiation, though Mickley et al. (1992) did find some protective action on mature behavioural responses when dizocilpine was administered prior to fractionated exposure of neonatal rats.

Though caution must be exercised in drawing conclusions from these results, it is interesting that the two compounds interfering with glutamate neurotransmission postsynaptically should both show a neuroprotective action subsequent to irradiation, suggesting a new indication for the glutamate antagonists as radiorestorative agents, a new category of radioprotective agents, for use after exposure has taken place.

References

Altman, J. and G.D. Das, 1965, Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats, J. Comp. Neurol. 124, 319.

Bassant, M. H. and L. Court, 1978, Effects of whole body gamma irradiation on the activity of rabbit hippocampal neurones, Radiat. Res. 75, 593.

Bayer, S.A. and J. Altman, 1974, Hippocampal development in the rat: cytogenesis and morphogenesis examined with autoradiography and low-level X irradiation, J. Comp. Neurol. 158, 55.

Bénavidès, J., C. Camelin, N. Mitrani, F. Flamand, F., A. Uzan, J. Legrand, C. Gueremy and G. Le Fur, 1985, 2-Amino-6-trifluoromethoxybenzothiazole, possible antagonist of excitatory amino acid neurotransmission. 2. Biochemical properties, Neuropharmacology 24, 1085.

Benoit, E. and D. Escande, 1991, Riluzole specifically blocks inactivated Na channels in myelinated nerve fibre, Pflügers Arch. 419, 603.

Bensimon, G., L. Lacomblez, V. Meininger and the ALS/riluzole study group, 1994, A controlled trial of riluzole in amyotrophic lateral sclerosis, New Engl. J. Med. 330, 585.

Chéramy, A., L. Barbeito, G. Godeheu and J. Glowinski, 1992, Riluzole inhibits the release of glutamate in the caudate nucleus of the cat in vivo, Neurosci. Lett. 147, 209.

Debono, M.W., J. Le Guern, T. Canton, A. Doble and L. Pradier, 1993, Inhibition by riluzole of electophysiological responses mediated by rate kainate NMDA receptors expressed in xenopus oocytes, Eur. J. Pharmacol. 235, 283.

Durand, R.E. and P.L. Olive, 1989, Radiosensitisation and radioprotection by BSO and WR-2721: the role of oxygenation, Br. J. Cancer 60, 517.

Gill, R. and J.A. Kemp, 1989, Protection of CA1 pyramidal cell function by MK-801 following ischemia in the gerbil, Neurosci. Lett. 105 101.

Graham, J.L., S.E. Smith, P.C. Pearce, A.G. Chapman and B.S. Meldrum, 1994, Effect of lamotrigine on amino acid concentrations after middle cerebral artery occlusion in the rat: a microdialysis and histological study, Abstracts of Symposium on Pharmacology of Cerebral Ischemia, Marburg, p. 41.

Gueneau, G., A. Privat, J. Drouet and L. Court, 1982, Subgranular zone of the dentate gyrus of young rabbits as a secondary matrix, Dev. Neurosci. 5, 345.

Halliwell, B., and J.M.C. Gutteridge, 1986, Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts, Arch. Biochem. Biophys. 246, 501.

Hebert, T., P. Drapeau, L. Pradier and R.J. Dunn, 1994, Block of the rat brain IIA sodium channel alpha subunit by the neuroprotective drug riluzole, Mol. Pharmacol. 45, 1055.

Hubert, J.P., J.C. Delumeau, J. Glowinski, J. Prémont and A. Doble, 1994, Antagonism by riluzole of entry ofcalcium evoked by NMDA and veratridine in rat cultured granule cells: evidence for a dual mechanism of action, Br. J. Pharmacol. 113, 261.

Lafon-Cazal, M., S. Pietri, M. Culcasi and J. Bockaert, 1993, Superoxide is responsible for NMDA-induced neurotoxicity in cultured cerebellar granule cells: electron paramagnetic resonnance spin trapping evidence, J. Neurochem. 61, S65C.

Leach, M.J., C.M. Marden and A.A. Miller, 1986, Pharmacological studies on lamotrigine. A novel potential antiepileptic drug: II Neurochemical studies on the mechanism of action, Epilepsia 27 490.

Leach, M.J., M.G. Baxter and M.A.E. Critchley, 1991, Neurochemical and behavioral aspects of lamotrigine, Epilepsia 32, 2 S4.

Martin, D., M.A. Thompson and J.V. Nadler, 1991, The neuroprotective agent riluzole inhibits release of L-glutamate and L-aspartate from slices of hippocampal area Ca1, Br. J. Pharmacol. 104 Suppl., P240.

Mickley, G.A., J.L. Ferguson and T.J. Nemeth, 1992, Serial injections of MK 801 (dizocilpine) in neonatal rats reduce behavioral deficits associated with X-ray-induced hippocampal granule cell hypoplasia, Pharmacol. Biochem. Behav. 43 785.

Mizoule, J., B. Meldrum, M. Mazadier, M. Croucher, C. Ollat, A. Uzan, J. Legrand, C. Gueremy and G. Le Fur, 1985, 2-amino 6-trifluoromethoxybenzothiazole, a possible antagonist of excitatory amino acid neurotransmission. 1. Anticonvulsant properties, Neuropharmacology 24, 767.

Oliver, C.N., P.E. Starke-Reed, E.R. Stadtman, G.J. Liu, J.M. Carney and R.A. Floyd, 1990, Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain, Proc. Natl. Acad. Sci. USA 87, 5144.

Park, C.K., D.G. Nehls, D.I. Graham, G.M. Teasdale and J. McCulloch, 1988, The glutamate antagonist MK-801 reduces focal cerebral ischemic brain damage in the rat, Ann. Neurol. 24, 543.

Pratt, J., J. Rataud, F. Bardot, M. Roux, J.-C. Blanchard, P.M. Laduron and J.M. Stutzmann, 1992, Neuroprotective actions of riluzole in rodent models of global and focal ischaemia, Neurosci. Lett. 140, 225.

Pellegrini-Giampetro, D.E., G. Cherici, M. Alesiani, V. Carla and F. Moroni, 1990, Excitatory amino acid release and free radical formation may cooperate in the genesis of ischemia-induced neuronal damage, J. Neurosci. 10, 1035.

Rataud, J., F. Debarnot, V. Mary, J. Pratt and J-M. Stutzmann, 1994, Comparative study of voltage-sensitive sodium channel blockers in focal ischaemia and electric convulsions in rodents, Neurosci. Lett. 172, 19.

Spence, A.M., K.A. Krohn, J.E. Steele, S.E. Edmondson and J.S. Rasey, 1988, WR-2721, WR-77913 and WR-3689 radioprotection in the rat spinal cord, Pharmacol. Ther. 39, 89.

Stutzmann, J-M and A. Doble, 1994, Blockade of glutamatergic transmission and neuroprotection: the strange case of riluzole, in: Neurodegenerative Diseases, eds. G. Jolles and J.M. Stutzmann (Academic Press, London) p. 205.

Wahl, F., M. Allix, M. Plotkine and R.G. Boulu, 1993, Effect of riluzole on focal ischaemia in rats, Eur. J. Pharmacol. 230, 209.

Wong, E.H.F., J.A. Kemp, T. Pristley, A.R. Knight, G.N. Woodruff and L.L. Iverson, 1986, The anti-convulsant MK-801 is a potent N-methyl-D-aspartate antagonist, Proc. Natl. Acad. Sci. USA 83, 7104.

Yuhas, J.M. and J.B. Storer, 1969, Differential chemoprotection of normal and malignant tissues, J. Natl. Cancer. Inst. 42, 331.