Effect of Riluzole on the Neurological and Neuropathological Changes in an Animal Model of Cardiac Arrest-Induced Movement Disorder

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ABSTRACT

Posthypoxic myoclonus and seizures precipitate as secondary neurological consequences in ischemic/hypoxic insults of the central nervous system. Neuronal hyperexcitation may be due to excessive activation of glutamatergic neurotransmission, an effect that has been shown to follow ischemic/hypoxic events. Therefore, riluzole, an anticonvulsant that inhibits the release of glutamate by stabilizing the inactivated state of activated voltage-sensitive sodium channels, was tested for its antimyoclonic and neuroprotective properties in the cardiac arrest-induced animal model of posthypoxic myoclonus. Riluzole (4–12 mg/kg i.p.) dose-dependently attenuated the audiogenic seizures and action myoclonus seen in this animal model. Histological examination using Nissl staining and the novel Fluoro-Jade histochemistry in cardiac-arrested animals showed an extensive neuronal degeneration in the hippocampus and cerebellum. Riluzole treatment almost completely prevented the neuronal degeneration in these brain areas. The neuroprotective effect was more pronounced in hippocampal pyramidal neurons and cerebellar Purkinje cells. These effects were seen at therapeutically relevant doses of riluzole, and the animals tolerated the treatment well. These findings indicate that the pathogenesis of posthypoxic myoclonus and seizure may involve excessive activation of glutamate neurotransmission, and that riluzole may serve as an effective pharmacological agent with neuroprotective potential for the treatment of neurological conditions associated with cardiac arrest in humans.

Myoclonus is a neurological disorder which is characterized by sudden, brief, shock-like involuntary movements caused by active muscle contractions or inhibitions (Fahn, 1986; Hallett, 1987). These symptoms can be precipitated by various pathological conditions affecting the central nervous system (CNS). In particular, posthypoxic myoclonus can occur after hypoxic and ischemic episodes (Fahn, 1986). The acute form of posthypoxic myoclonus is most often associated with seizures and occurs spontaneously. It is a life-threatening condition that requires immediate medical treatment (Wijdicks et al., 1994). The delayed form of posthypoxic myoclonus is stimulus-sensitive; the muscle jerks are expressed as a type of action myoclonus. This condition affects motor function to different degrees of severity ranging from mild to serious debilitation. Therapeutic treatment for posthypoxic myoclonus has been limited, mainly due to the lack of a clear understanding of the pathophysiological mechanisms involved. Lack of a suitable animal model has hindered both the understanding of biochemical mechanisms underlying the disorder and better clinical management of the resulting symptoms. Recently, we have developed an animal model of posthypoxic myoclonus by inducing 8- to 10-min cardiac arrest in rats (Truong et al., 1994; Kanthasamy et al., 1996b). This animal model exhibits behavioral and pharmacological characteristics that resemble those found in human posthypoxic myoclonus (Jaw et al., 1994; Truong et al., 1994; Matsuzato et al., 1995a,b; Truong et al., 1995; Kanthasamy et al., 1996a;b; for review: Kanthasamy et al., 1996b). Currently, it is the most realistic animal model available to study pathophysiological mechanisms and to develop novel pharmacotherapies for the treatment of this neurological disorder.

The precise mechanisms underlying posthypoxic myoclonus are not clearly understood, although imbalances in one or more neurotransmitter systems seem to be involved (Matsuzato et al., 1995a,b; Kanthasamy et al., 1996a,b). Gluta-
mate acts as a major excitatory neurotransmitter in the CNS where it mediates fast, excitatory synaptic neurotransmission, and it may play a role in neuronal communication and CNS pathology (Garthwaite and Meldrum, 1990; Lipton and Rosenberg, 1994). Excessive glutamatergic neurotransmission may be due to an increase in extracellular glutamate, an effect that has been shown to follow ischemic/hypoxic events (Butcher et al., 1990; Globus et al., 1991). Overactivity of glutamatergic neurotransmission can lead to rapid changes in neuronal biochemistry that culminate in excitotoxic cell death in susceptible neuronal populations (Choi and Rothman, 1990). Thus, excitatory amino acid-mediated neuronal overexcitation is thought to be an underlying pathological mechanism both in neuronal damage and in the neurological consequences of the posthypoxic state. In this context, we have previously shown that N-methyl-D-aspartate (NMDA) receptor antagonists (Matsumoto et al., 1995b) and nitric oxide synthase inhibitors are effective in attenuating posthypoxic myoclonus (Truong et al., 1995), indicating that the glutamate system may play a crucial role in the pathophysiology of this disorder.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) is an anticonvulsant that primarily intervenes with glutamate-mediated excitation by stabilizing voltage-dependent sodium channels in their inactivated configuration (Herbert et al., 1994; Doble, 1996). In addition to its anticonvulsant properties, riluzole exhibits neuroprotective action in various in vitro and in vivo models (Malgouris et al., 1989; Pratt et al., 1992; Dessi et al., 1993; Estevez et al., 1995). This drug has been shown to prevent anoxic injury in cultured cerebellar neurons (Dessi et al., 1993), reduce glutamate neurotoxicity in motoneurons (Estevez et al., 1995), and act as a neuroprotectant in rodent models of cerebral ischemia (Malgouris et al., 1989; Pratt et al., 1992). It has also been recently approved for treatment of amyotrophic lateral sclerosis, a progressive motor neuron disease thought to involve hyperglutamatergic neurotransmission (Lacomblez et al., 1996). Because of its therapeutic potential in glutamate-mediated overexcitation, we have tested the antymyoclonic and neuroprotective effects of riluzole in the posthypoxic animal model, using both behavioral and histological measures. Also, we have examined the utility of Fluoro-Jade histochemistry, a novel histological method, for neuroprotective studies in the cardiac arrest model.

**Materials and Methods**

**Animals.** Adult male Sprague-Dawley rats (200–250 g; Zivic-Miller Laboratories, Inc., Alison Park, PA) were used in these experiments. The rats were housed two per cage in a temperature-controlled room (23°C) with a 12:12-h light/dark cycle. The animals were allowed free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California Irvine, and Memorial Health Services.

**Animal Model of Cardiac Arrest-Induced Posthypoxic Myoclonus.** The cardiac arrest procedure was performed as described previously (Kanthasamy et al., 1996a,b). Briefly, each rat was anesthetized with ketamine (85 mg/kg i.p.) and xylazine (15 mg/kg i.p.). Atropine (0.04 mg/kg i.p.) was administered to minimize respiratory secretion. Methoxyflurane was provided as supplemental anesthesia if necessary. The trachea was intubated with an 18-gauge catheter, which was then attached to a ventilator (settings: 425 ml/min N2O, 175 ml/min O2, 60 strokes/min, 5 cm of H2O positive end-expiratory pressure). The rat was placed on a heating pad, and electrocardiogram electrodes were attached. Body temperature was maintained at 37 ± 0.5°C with a rectal temperature probe controlled by a servo-feedback circuit. The left femoral artery and vein were catheterized to monitor arterial blood pressure and for the administration of drugs, respectively. Cardiac arrest was initiated and maintained by mechanically obstructing all the major blood vessels, including the aorta, by hooking them with an L-shaped loop and simultaneously compressing the chest. Cessation of ventilation and drop in blood pressure were confirmed by electrocardiogram and blood pressure tracings monitored on the polygraph. Resuscitation began at 8 min after the cardiac arrest by resuming ventilation (100 strokes/min, 100% O2), manual thoracic compressions, and i.v. injection of 10 μg/kg epinephrine and 4 μg/kg sodium bicarbonate. Upon successful resuscitation, each rat was weaned from the ventilator, the catheters were removed, and wounds were sutured. The animal was placed in an oxygen tent on a heating pad until it completely recovered from the surgical coma. Normally, the animals recovered from surgery and began feeding themselves 3 to 5 h post surgery. This cardiac arrest procedure yielded a survival rate of approximately 90% with minimal postoperative care.

**Riluzole Treatment.** The cardiac-arrested rats were divided into four groups receiving 0, 4, 8, or 12 mg/kg riluzole in 0.01 N hydrochloric acid, i.p. This dose range was selected because it has been shown to be effective in other animal studies (Pratt et al., 1992; Doble, 1996). Three doses of riluzole were administered at separate times. The first dose was given 2 h after the resuscitation, the time at which the animals started recovering from the cardiac arrest. The second dose was given 24 h after the cardiac arrest procedure and the seizure score was estimated 15 min after the administration of the second dose. The third dose was given 15 min before evaluation of audiogenic myoclonus, which was evaluated 48 h after surgery. In separate experiments, just the first dose of riluzole (12 mg/kg) was administered to determine the efficacy of single dose treatment. Also, the first dose was eliminated, whereas the subsequent doses were given to elucidate whether neurodegeneration was due to the anti-ischemic or antisepseur properties of riluzole.

**Behavioral Testing.** After 24 h of postsurgical recovery, the posthypoxic rats were tested for seizure activity. Either drug or vehicle was injected, and a quantitative measure of seizure activity was determined (Truong et al., 1995) for each rat by a blinded observer using the following rating scale: 0, no seizure; 1, running only/no convulsion; 2, running phase with generalized clonus involving forelimbs, hindlimbs, pinnae, and/or vibrissae; 3, tonic flexion of neck, trunk, and forelimbs; 4, convulsion with complete tonic extension of hindlimbs; and 5, maximal convulsion followed by loss of consciousness.

The rats were tested for auditory stimulus-induced myoclonus as previously described (Truong et al., 1994; Kanthasamy et al., 1996a,b) 24 h after seizure testing. Rats were placed in clear Plexiglas cages (44 × 22 cm) at least 10 min before the behavioral testing and were then presented with 45 clicks (95 dB, 0.75 Hz, 40 ms) of a metronome stimulus. The involuntary muscle jerks to each click were scored based on the following criteria: 0, no jerks; 1, ear twitch; 2, ear and head jerk; 3, ear, head, and shoulder jerk; 4, whole body jerk; and 5, whole body jerk of such severity that it caused a jump. The cumulative score of 45 clicks yielded the total myoclonus score for each animal. The myoclonus scores for each animal were determined at 15, 30, 60, 120, and 180 min after administration of the drug or vehicle, and were compared to baseline scores determined for each animal before the surgical procedure.

**Histological Studies.** After the behavioral studies, the rats were injected with a lethal dose of sodium pentobarbital (100 mg/kg i.p.) and perfused intracardially with saline followed by 10% Formalin in 0.1 M phosphate buffer (pH 7.4). The rats were then decapitated, and their brains were removed and immersed in Formalin solution to further fix the brain tissue. The brains were then embedded in paraffin wax. Serial coronal sections (6-μm thickness) were taken...
from various sections of the brain, stained for Nissl substance using cresyl violet, and examined for pathological changes.

Similar sections were alternatively stained with Fluoro-Jade, a newly developed fluorescent marker that selectively stains degenerating neurons (Schmued et al., 1997; Freyaldenhoven et al., 1997). Briefly, slides containing sections of brain tissue were dewaxed in xylene, immersed in 100% ethanol for 5 min, 70% alcohol for 2 min, and then rinsed with two 1-min changes of dd-H2O. The slides were then incubated with freshly prepared 0.06% potassium permanganate for 17 min and rinsed again with two 1-min changes of dd-H2O. This was followed by a 30-min incubation in 0.001% Fluoro-Jade solution at room temperature and two 1-min rinses with dd-H2O. The slides were air dried with a blow dryer for 10 min at low heat, placed in xylene for 2 min, covered with Permount, and stored in a dark area. The sections were viewed under a fluorescence research microscope (model BH2; Olympus) using a fluorescein-5-isothiocyanate filter.

Quantitative Histological Analysis. The image processing and quantitative analysis of histological data were performed using Image-Pro plus software (Media Cybernetics, Inc., Silver Spring, MD) in combination with a SPOT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI). The images acquired from the slides representing the specific color signals (violet for cresyl violet and green fluorescence for Fluoro-Jade) were compared with the slides that were devoid of the staining (reagent-blank slides) to construct a Magro algorithm with specific hues. The cells represented by the selected color were automatically defined, encircled, numbered, and measured.

Statistical Analysis. Data were expressed as mean ± S.E.M. and statistical significance was determined by ANOVA with the Dunnett’s test in the case of multiple comparisons or with Student’s t-test in case of simple comparisons. Differences were accepted as significant at p < .05 or less.

Results

Cardiac Arrest-Induced Neurological Deficits. The cardiac arrest-induced posthypoxic myoclonus rat serves as a suitable experimental model for the present study, because this model exhibits not only substantial reproducible neuro-pathological changes but also behaviorially expresses the neurological syndrome. After cardiac arrest, the rats exhibited spontaneous seizures within 12 h and then showed audiogenic seizures for the next 24 to 48 h. The types of seizures generated after cardiac arrest were tonic, partial with wild running behavior, and generalized clonic-tonic with loss of consciousness. After the seizure period, the rats exhibited stimulus-sensitive action myoclonus that persisted for a relatively longer period, up to 3 weeks postsurgery.

Antiepileptic Effect of Riluzole in Cardiac Arrest-Induced Posthypoxic Myoclonus Animal Model. Because postanoxic seizure precipitates as an early neurological symptoms of severe ischemic insults, antiepileptic properties of riluzole were evaluated in the audiogenic seizures phase of the posthypoxic animal model of myoclonus. Riluzole dose-dependently reduced the audiogenic seizure in the cardiac arrest animals (Fig. 1). At a dose of 8 mg/kg, the seizure score was significantly (p < .05) reduced, whereas at a higher dose of 12 mg/kg, the drug almost completely blocked seizure activity (p < .01). Also, riluzole treatment reduced the incidence of the development of spontaneous seizures, which occurs 12 to 24 h postsurgery (data not shown).

Antimyoclonic Effect of Riluzole in Cardiac Arrest-Induced Posthypoxic Myoclonus Animal Model. To determine the antimyoclonic properties of riluzole, the drug was tested for its ability to attenuate stimulus-sensitive myoclonus. Riluzole dose-dependently attenuated myoclonus in the posthypoxic rats as shown in Fig. 2. Significant reductions (p < .05) in myoclonus scores were seen at dosages of 8 and 12 mg/kg within 30 min of injection. The rats showed behavioral improvements up to 30 min postinjection, after which the animals exhibited relatively stable behavior. At the 4-mg/kg dose, the myoclonus score dropped initially, but returned to levels similar to control levels within 1 h. Importantly, riluzole at these doses (4–12 mg/kg, i.p.) did not cause any noticeable behavioral side effects. Such side effects are commonly a problem with drugs that interfere in glutamatergic transmission (Koek and Colpaert, 1990; Carter, 1994). In addition, unlike NMDA antagonists (e.g., MK-801), the drug treatment did not markedly alter the body temperature (baseline = 36.3°C ± 0.6°C versus riluzole (12 mg/kg) treatment = 35.9°C ± 0.7°C).

To determine the efficacy of a single dose of riluzole, 12 mg/kg (i.p.) was tested. As shown in Fig. 3, a single dose of 12 mg/kg riluzole treatment 2 h after cardiac arrest surgery did not significantly attenuate anoxic seizures (A) and posthypoxic myoclonus (B). This single dose treatment showed only
Riluzole Attenuates Posthypoxic Neurological Deficits

Effect of a single dose of riluzole on the posthypoxic myoclonus. Rats were administered either vehicle or riluzole (12 mg/kg i.p.) 2 h after the cardiac arrest surgery. Audiogenic seizure (A) and myoclonus score (B) measured 24 and 72 h after cardiac arrest, respectively. Data represent mean ± S.E.M of six animals in each group.

Neuroprotective Effect of Riluzole on Hippocampal Pyramidal and Cerebellar Purkinje Neurons in Posthypoxic Myoclonus Model. To determine the neuroprotective effect of riluzole against posthypoxic neuronal damage, the brains were histologically evaluated after the behavioral measurements. Representative photographs of histological changes in brain sections containing dorsal hippocampus stained with cresyl violet are presented in Fig. 4. Control animals exhibited normal cellular architecture of pyramidal cells in hippocampus (Fig. 4, A and B). Hippocampal sections of posthypoxic rats treated with vehicle showed a severe loss of the pyramidal cells in region CA1 and CA2, as well as losses in the dentate granule neurons (Fig. 4, C and D). Damage to CA1 neurons was more dramatic than that of other regions. Systemic injection of riluzole (12 mg/kg i.p.) almost completely blocked the neuronal damage to pyramidal cells of the hippocampus (Fig. 4, E and F). In Fig. 5, control animals show normal morphological characteristic of Purkinje cells as stained with cresyl violet (Fig. 5A). These are the round bipolar cells that lie between the molecular and granule layers. Cardiac arrest caused almost complete loss of Purkinje cells (Fig. 5B). Riluzole treatment dramatically rescued the Purkinje cells from cardiac arrest-induced ischemic injury (Fig. 5C). There was no clear cell loss noted in the other areas of the brains stained with cresyl violet.

Figure 6 summarizes the quantitative data analysis of cresyl violet-stained cells in the hippocampus (A) and cerebellum (B) of riluzole-treated rats. Cardiac arrest reduced the hippocampal pyramidal cells and cerebellar Purkinje cells to 6 and 21% of noncardiac arrested rats, respectively. Riluzole treatment dose-dependently attenuated the neuronal damage. At the 12-mg/kg dose, riluzole protected 80% (p < .01) of neuronal loss in both hippocampus and cerebellum.

We have also evaluated the neuroprotective efficacy of riluzole by eliminating the initial dose (2 h postcardiac arrest). This experiment was conducted to determine whether the neuroprotective effect of riluzole is related to its anti-ischemic or anticonvulsant properties. The cardiac-arrested rats that received riluzole treatment (12 mg/kg i.p.) only at 24 and 48 h postarrest were not protected from ischemic neuronal injury either in the hippocampus or cerebellum. The quantification of neuronal damage by cresyl violet-staining showed a hippocampal pyramidal neuronal loss of 93 ± 6% and the cerebellar Purkinje neuronal loss of 78 ± 5% in the vehicle-treated group. In comparison, the riluzole-treated group showed a 90 ± 7% loss of pyramidal neurons in the hippocampus and 81 ± 8% Purkinje cell loss in the cerebellum.

Usefulness of Fluoro-Jade Histochemistry for Neuroprotective Studies in Cardiac Arrest-Induced Posthypoxic Myoclonus Model. The Fluoro-Jade histochemistry technique stained only degenerating neurons indicated by a bright fluorescence and was remarkably helpful in identifying defective cells in a given population (Schmued et al., 1997). This technique more clearly localizes damaged neurons, especially in the cerebellum, as compared to the Nissl staining. A representative Fluoro-jade labeling in the hippocampus and cerebellum is presented in Figs. 7 and 8, respectively. No Fluoro-jade positive fluorescence staining was noted in either hippocampal (Fig. 7A) or cerebellar regions (Fig. 8A) of control animals. Hippocampal and cerebellar sections from the posthypoxic rats showed many Fluoro-Jade positive cells in the pyramidal (Fig. 7B) and Purkinje cell layers (Fig. 8B), whereas a very few or no such positive cells were seen in similar sections of the riluzole-treated posthypoxic rats (Figs. 7C and 8C). Although the loss of cells in hippocampus and cerebellum was quite obvious with cresyl violet staining, this technique showed subtle or no morphological changes in many areas that clearly showed neuronal damage with Fluoro-Jade. These areas include the parietal cortex, hind limb cortex, thalamus, indusium griseum, and zona incerta (data not shown). In addition, Fluoro-Jade staining displayed degenerating axons and dendrites that were not seen with cresyl violet stain.

The quantitative analysis of Fluoro-jade positive cells is presented in Fig. 9. Riluzole afforded a significant (p < .01) neuroprotection in both the hippocampus (Fig. 9A) and cerebellum (Fig. 9B) as compared with vehicle-treated groups. The effect was dose-related; however, the neuroprotective effect of riluzole as seen by this stain was more dramatic in the cerebellum than in the hippocampus.
Discussion

The experimental results indicate that riluzole has significant antimyoclonic and antiepileptic effects in cardiac arrest-induced movement disorders, and that the drug offers neuroprotection at the cellular level from posthypoxic degenerative mechanisms as seen by staining with cresyl violet and Fluoro-Jade reagent. The antimyoclonic action of riluzole is readily achieved at therapeutic doses and the effect is dose-dependent, indicating the pharmacological validity of the study. Riluzole is an antiexcitotoxic agent that has been shown to be effective in both in vitro and in vivo models of glutamate overactivation (Malgouris et al., 1989; Benoit and Escande, 1991; Estevez et al., 1995). Together, the present study suggests that excessive activation of glutamatergic neurotransmission after an ischemic insult may play a role in the posthypoxic myoclonus and seizure, and attenuation of this neuronal hyperexcitability by pharmacological means may be beneficial for treating the neurological and neuropathological changes associated with cardiac arrest.

The mechanism(s) by which riluzole confers its antimyoclonic effect and neuroprotection is not precisely known. However, previous studies (Martin et al., 1993; Herbert et al.,

Fig. 4. Neuroprotective effect of riluzole on hippocampal sections as seen by staining with cresyl violet. A, dorsal hippocampus in control rats appears histologically normal. B, the cell bodies of hippocampal pyramidal neurons in CA1 region are densely distributed. C, dorsal hippocampus of cardiac arrested rats shows severe loss of cells. D, there is an almost complete degeneration of pyramidal neurons in CA1 region. E, dorsal hippocampus of cardiac arrested rat treated with riluzole (12 mg/kg i.p.) shows a remarkable neuroprotective effect. F, the riluzole treatment has almost completely blocked the cardiac arrest-induced neuronal degeneration in pyramidal cells. Original magnification, A, C, and E = 40×; B, D, and F = 100×.
Doble, 1996) have revealed that riluzole may mediate antiexcitatory amino acid properties via one or more of the following mechanisms: 1) inhibition of glutamate release from presynaptic glutamatergic nerve terminals, 2) stabilization of voltage-dependent sodium channels in their inactive conformation, 3) noncompetitive blockade of postsynaptic NMDA ionotropic channels, and 4) activation of a G protein-dependent pathway. The first two mechanisms appear to be interrelated in terms of synaptic transmission because a prolonged inactivation of sodium channels will reduce the ability of the cells to depolarize (firing rate) and thereby block the release of glutamate. Previous studies have shown that riluzole inhibits the release of glutamate both in vitro and in vivo (Cheramy et al., 1992; Martin et al., 1993). In addition, both glutamate release and sodium channel inactivation are blocked by pertussis toxin (Hubert et al., 1994), suggesting that activation of a G protein-related mechanism may also play a role in the biological effect of riluzole. Radioligand-binding studies do not support the notion that riluzole inhibits glutamate receptors because the drug does not bind to NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, or metabotropic receptors (Benoit and Escande, 1991).

In global cerebral ischemia, glutamate release occurs during the occlusion period; however, the levels return to baseline in the reperfusion period. In our cardiac arrest model, which resembles the global ischemic model, glutamate release probably occurs during the cardiac arrest period. Because the first dose of riluzole was administered 2 h postresuscitation, riluzole probably does not act by the inhibition of the acute release of glutamate, but rather by attenuation of delayed accumulation of synaptic glutamate through inhibition of activated sodium channels in the posthypoxic state. The loss of cellular bioenergetics and ionic homeostasis are early events of ischemia/hypoxia. Na\(^+\) influx initiates the cellular depolarization that contributes to deregulation of a number of energy-dependent physiological events, including ionic homeostasis and neurotransmitter reuptake. It has been suggested that the beneficial effect of Na\(^+\) channel blockers could be the consequence of a reduction in energy demand resulting in improved resistance to ischemia and preservation of Ca\(^{++}\) homeostasis (Urenjak and Obrenovitch, 1996). There is also substantial evidence indicating that increased levels of synaptic glutamate in the posthypoxic state.
poxic period is primarily mediated by reversal of the Na\(^+\)-dependent glutamate transporter rather than by the vesicular release (Taylor et al., 1995). Therefore, blockade of the Na\(^+\) channel subsequently restores the levels of glutamate and bioenergy, and thereby mitigates excitotoxic signaling cascades (activation of proteases, phospholipases, kinases, nitric oxide synthase, etc.) that otherwise lead to neuronal damage.

A recent patch-clamp study demonstrates that riluzole, at the therapeutic concentration, selectively inhibits the inactivated state of activated sodium channels through a use-dependent fashion (Herbert et al., 1994). Because of this, the normal sodium channels are insensitive to the inhibitory effect of riluzole. Thus, it appears that riluzole’s preferential affinity for the activated Na\(^+\) channel during cardiac arrest prevents excessive neuronal firing only in susceptible cells without disturbing the synaptic transmission in normal cells. This offers a favorable therapeutic index as compared to conventional sodium channel inhibitors that block both open and closed channels. To further confirm the effect of riluzole on Na\(^+\) channels, we tested lamotrigine, another anticonvulsant that mediates its pharmacological action through Na\(^+\) channel inhibition (Cheung et al., 1992; Xie et al., 1995).

Under a similar treatment paradigm, lamotrigine attenuated neurological and neuropathological changes in the cardiac arrest model at the high doses (A.K., T. Tith, B. Nguyen, A. Tron, D. Truong, submitted). However, at the higher dose (25 mg/kg i.p.) the drug produced behavioral side effects including motor uncoordination and hypotonia. These side effects may be due to lack of selectivity for activated Na\(^+\) channels over closed channels at the higher doses (Leach et al., 1986). Recent studies have shown that many of a new class of anticonvulsants that block Na\(^+\) channels possess neuroprotective properties in stroke and traumatic head injury models (Taylor and Meldrum, 1995). Taken together, the selective inactivation of activated sodium channels may account for the observed neuroprotective effects of riluzole in cardiac arrest-induced posthypoxic myoclonus model without deleterious side effects.

The ability of riluzole to prevent neuronal degeneration in both the hippocampal pyramidal cells and cerebellar Purkinje cells in our animal model reflects the potent neuroprotective potential of the drug on diverse neuronal populations. Riluzole’s neuroprotective properties have also been seen in the gerbil model of global ischemia, in which this drug was found to block necrosis of pyramidal cells in the CA1 region (Malgouris et al., 1989; Pratt et al., 1992). Because the limbic system plays a crucial role in epileptogenesis, the neuroprotective effect of riluzole in the hippocampus can be attributed to the observed anticonvulsant properties of the drug. In the cerebellum of posthypoxic rats, degeneration of Purkinje cells made evident by the histological analyses indicate that pathways between sensory inputs to the cerebellar cortex and deep cerebellar and lateral vestibular nuclei may be disrupted; these events could cause dysfunctional neurotransmission to motor nuclei and subsequent impairment of motor control (Guyton, 1991). Thus, the ability of riluzole to block excessive glutamatergic neurotransmission in the hippocampus and cerebellum may contribute to the overall prophylactic action of the drug against pathological motor function after ischemic or hypoxic insult.

The clinical potential for riluzole for use in treating posthypoxic myoclonus is enhanced by its lack of effect on cardiovascular activity and its few side effects compared with other antiglutamatergic agents. The lack of cardiovascular effect may be attributed to the insensitive nature of low frequency Na\(^+\) channels in the cardiac cells to riluzole (Doble, 1996). An implication of the present study is that administration of riluzole after 2 h postcardiac arrest may provide an excellent therapeutic window for treatment of neuronal damage and behavioral deficits after severe cardiac arrest.

The present study demonstrates that the initial 2-h dose is crucial to obtain a neuroprotective effect in the cardiac arrest model. This emphasizes the importance of the initial avail-
ability of the neuroprotective agent in the critical onset phase of the ischemic neurodegenerative process. This result also suggests that the neuroprotective effect of riluzole may primarily be due to the anti-ischemic properties of the drug. Another advantage of using riluzole for treatment of posthypoxic neurological consequences is that the drug is orally active and readily crosses the blood-brain barrier. Furthermore, its marked ability to attenuate myoclonus in rats at dosages well tolerated in humans (Lacomblez et al., 1996) augments its potential for therapeutic use in the treatment of human forms of this disorder. Because of its favorable pharmacokinetic and pharmacodynamic properties, the drug has recently been approved for treatment of progressive neurodegeneration in amyotrophic lateral sclerosis, a neurological disease in which overactivity of the glutamate system has been well documented (Leigh and Meldrum, 1996). Although several antiglutamatergic agents have been shown to be effective in attenuating neuronal in damage animal models of ischemic/hypoxic neuronal injury (Boast et al., 1988; Faden et al., 1989), these drugs produce severe contraindications, including psychotomimetic effects, learning deficits, and motor uncoordination (Koek and Colpaert, 1990; Carter, 1994). In addition, several of these compounds do not cross the blood-brain barrier very effectively, posing a problem in administering these drugs in clinical settings.

In conclusion, this study demonstrates that riluzole dramatically reduces neurobehavioral and histological changes in the cardiac arrest-induced animal model of posthypoxic myoclonus. It also suggests that excessive activation of neuronal excitation may play an important role in the pathogenesis of posthypoxic myoclonus, and that riluzole may provide a therapeutic benefit in the medical management of neurological complications associated with cardiac arrest.

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