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Prodystonic effects of riluzole in an animal model of idiopathic dystonia related to decreased total power in the red nucleus?

Angelika Richter *, Manuela Gernert, Wolfgang Löscher

Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, D-30559 Hannover, Germany

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Abstract

The effects of riluzole (2-amino-6-trifluoromethoxy benzothiazole) on the severity of dystonia were examined in mutant hamsters (dt^{sz}), an animal model of idiopathic dystonia in which dystonic attacks can be age dependently induced by mild stress. Previous studies in hamsters have shown antidystonic activity of various glutamate receptor antagonists whereas lamotrigine, considered as an inhibitor of glutamate release, exerted prodystonic effects. The latter, unexpected, finding prompted us to investigate riluzole which is thought to possess antiglutamatergic properties with mechanisms similar to those of lamotrigine. Riluzole (2, 5, 10 or 20 mg/kg i.p.) dose dependently decreased the latency to onset of dystonic attacks. A dose of 10 or 20 mg/kg significantly increased the severity of dystonia. Even in dt^{sz} hamsters older than 70 days, i.e., after spontaneous remission of age-dependent dystonia, riluzole (10 or 20 mg/kg) provoked severe long-lasting (> 4 h) dystonic attacks. At a dose of 20 mg/kg, riluzole provoked short-lasting (< 1 h) dystonic disturbances also in non-dystonic control hamsters. Electroencephalographic recordings from depth electrodes in the red nucleus, where recent studies have shown abnormal neural activity before and during dystonic attacks in dt^{sz} hamsters, revealed that riluzole (10 mg/kg) tended to cause a further decrease of the total power in dt^{sz} hamsters and significantly reduced the total power in control animals. This finding may indicate that the prodystonic effects of riluzole are related to alterations of rubrospinal activity. With regard to antidystonic effects of glutamate receptor antagonists demonstrated in previous studies, the prodystonic effects of riluzole and, as shown by recent experiments, of lamotrigine also, may be due to the lack of selectivity of these drugs to inhibit glutamate release. © 1997 Elsevier Science B.V.

Keywords: Glutamate; Na^+ channel blocker; Movement disorder; Paroxysmal choreoathetosis

1. Introduction

Overactivity of excitatory pathways within the basal ganglia has been suggested to play an important role in the pathogenesis of movement disorders, such as Parkinson's disease, Huntington's disease, dyskinesia and drug-induced dystonia (DeLong, 1990; Graham et al., 1994). Therefore, the pharmacological modulation of excitatory amino acid transmission in the basal ganglia may be of interest for the treatment of these disorders (Pisani et al., 1995). Antiglutamatergic compounds, such as lamotrigine and riluzole (2-amino-6-trifluoromethoxy benzothiazole; RP 54 274), which both inhibit glutamate release, may provide a novel approach for the treatment of movement disorders (Brodie, 1992; Zipp et al., 1993, 1995; Boireau et al., 1994). Although the pathomechanisms of idiopathic dystonia, a neurological syndrome characterized by sustained muscle contractions causing twisting movements or abnormal postures, are largely unknown (McGeer and McGeer, 1988), beneficial effects of NMDA receptor antagonists may indicate that abnormal activity of the glutamatergic system is pathophysiologically involved in dystonia (Mundinger and Milios, 1985; Richter et al., 1991).

Previous pharmacological studies in mutant dystonic hamsters (genetic symbol dt^{sz}), an animal model of idiopathic paroxysmal dystonia, revealed antidystonic effects of various glutamate receptor antagonists (Richter et al., 1991, 1993; Löscher and Richter, 1993). Since glutamate receptor antagonists are either toxic or currently not available for clinical use in movements disorders, we recently tested lamotrigine in dt^{sz} hamsters. Lamotrigine is a well-tolerated novel antiepileptic drug which is thought to act

* Corresponding author. Tel.: (49-511) 953-8723; Fax: (49-511) 953-8581; e-mail: arichter@pharma.tiho-hannover.de

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functionally as a glutamate antagonist by inhibition of presynaptic glutamate release (Leach et al., 1986). However, instead of the expected antidystonic efficacy, lamotrigine (30 mg/kg i.p.) aggravated dystonia in mutant hamsters and even provoked dystonic disturbances in genetically related non-dystonic control hamsters of an inbred line, but not in non-related control hamsters of an outbred line (Richter et al., 1994). This unexpected finding, possibly explained by the lack of selectivity of lamotrigine to inhibit glutamate release (Richter et al., 1994), deserved further investigation. Therefore, in the present study the effects of riluzole were examined in mutant dystonic hamsters, because riluzole shows comparable mechanisms of action as lamotrigine (Leach et al., 1986; Lang et al., 1993; Hebert et al., 1994; Song et al., 1996).

Riluzole, a novel neuroprotective agent for amyotrophic lateral sclerosis with anticonvulsant and anti-ischemic properties, has been considered to act by inhibition of glutamate/aspartate release (Martin et al., 1993). Similarly to that of lamotrigine, the effect of riluzole on synaptic transmission seems to be mediated, in part, by stabilization of presynaptic neurones through inhibition of voltage-activated sodium currents (Benoit and Escande, 1991; Hebert et al., 1994; Umemiya and Berger, 1995). Riluzole inhibits the release not only of glutamate (at 10–100 μM) but also of γ-aminobutyrate (GABA) in hippocampus slices of rats (at 100 μM; Martin et al., 1993). This compound has also been found to inhibit spontaneous serotonin and dopamine release in rat striatum (Cheramy et al., 1986; Becquet et al., 1990). Furthermore, riluzole suppresses glycinergic synaptic transmission in motoneurons of rats, probably by a presynaptic action (Umemiya and Berger, 1995). Riluzole has, moreover, been reported to induce a dose-dependent decrease in the uptake of dopamine, GABA and glutamate into striatal synaptosomes, which may be related to the block of sodium channels (Samuel et al., 1992).

Neurochemical and electrophysiological studies in mutant hamsters have shown alterations within the striatum, thalamic nuclei, cerebellum and red nucleus (Nobrega et al., 1995, 1996; Richter et al., 1995; Gernert et al., 1997), i.e., regions which have been implicated in idiopathic dystonia in humans (McGeer and McGeer, 1988). Recent 2-deoxyglucose uptake studies showed a most dramatic increase of 159% above control in the red nucleus during dystonic attacks in mutant hamsters (Richter et al., 1995), whereas electroencephalic (EEG) recordings with depth electrodes have shown a decrease of total power in the red nucleus before and during dystonic attacks in this animal model (Gernert et al., 1997). Although it seems unlikely that the red nucleus is the primary focus of dystonia, the previous findings suggest an abnormal output to the spinal cord via the red nucleus in dystonic hamsters.

The aim of the present study was to investigate whether riluzole exerts prodystonic effects, similar to recent findings with lamotrigine (Richter et al., 1994). In addition, quantitative EEG recordings from depth electrodes in the red nucleus were undertaken in order to examine if prodystonic effects of riluzole, shown by the present study, could be related to a further decrease of the total power in this output nucleus to the spinal cord.

2. Methods

2.1. Animals

The experiments were carried out in 4 groups of 7–11 mutant dystonic inbred hamsters, 2 groups of 5–6 non-dystonic inbred hamsters and 2 groups of 7–8 non-dystonic hamsters of an outbred line. The two inbred lines of Syrian hamsters, i.e., mutant dystonic hamsters in which dystonia is transmitted by an autosomal recessive gene (genetic symbol dt^{sz}) and related non-dystonic (control) hamsters were obtained by selective breeding, as described in detail elsewhere (Fredow and Löscher, 1991). The non-dystonic control hamsters of the inbred line used in this study were obtained by crossing heterozygous hamsters of the dystonic line with a normal LVG (Lakeview Golden) strain of hamsters and using the offspring, which did not carry the gene for phenotypic motor disturbances (as determined by backcrossing) for further breeding (see Fredow and Löscher, 1991). In addition, (non-dystonic) Syrian hamsters of an outbred line were used, obtained by breeding pairs which were provided by a commercial breeder (Central Institute for Laboratory Animal Breeding, Hannover). Neither the non-dystonic hamsters of the inbred line nor the genetically different control hamsters of the outbred line exhibit spontaneous or stress-induced dystonic disturbances.

In dt^{sz} mutant hamsters, characterized as an animal model of paroxysmal dystonia in previous clinical, electrophysiological and pharmacological studies (Löscher et al., 1989), attacks of generalized twisting movements and abnormal postures occur in the absence of histopathological alterations in the brain or spinal cord (Wahnschaffe et al., 1990) similar to idiopathic dystonia in humans. The severity of dystonia shows an age-dependent time-course with maximum severity between the 30th and 40th day of life (max-period, suitable for study of antidystonic effects of drugs). Thereafter, the severity of dystonia slowly declines (post-max period, suitable to examine prodystonic drug effects) until complete remission of dystonia occurs at the age of about 70 days (Richter and Löscher, 1993).

2.2. Drug testing

In dt^{sz} mutant hamsters dystonic attacks can be induced by a 'triple stimulation technique' (Löscher et al., 1989), i.e., (1) taking the animal from its home cage and placing it on a balance, (2) i.p. injection of vehicle (pre- and post-drug controls) or of the drug (i.e., in the present study

of riluzole), respectively, and (3) placement of the hamster in a clean and empty plastic cage (one animal per cage). Prior to pharmacological examinations, the hamsters were tested with the triple stimulation procedure every 2 to 3 days, starting at the age of weaning (21st day of life) until reproducible stages and latencies to onset of dystonia were recorded. Since the individual maximum stage of dystonia is usually reached within 3 h after triple stimulation, the hamsters had to be observed for 3 h and the severity of dystonia and the latencies to the different stages were noted. The severity of dystonia was rated with the following score system: stage 1, flattened ears and flattened posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with retarded setting of the forepaws; stage 3, stiffened hindlimbs so that the animals appear to walk on tiptoe in a dysmetric hypergait; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilization in a twisted, hunched posture with hind- and forelimbs tonically extended forward, Straub tail, alternating unilateral forelimb elevation, opisthotonus. After reaching the individual maximum stage the hamsters usually recover within 2–5 h. The control trials were undertaken two days before (pre-drug) and two days after (post-drug) drug testing. All control and drug trials in dt^{sz} mutant hamsters and in age-matched control hamsters of the inbred or outbred line were done at the same time of the day, e.g., between 9.00 and 12.00 a.m.

Cataleptic effects of riluzole were determined by quantifying descent latency in a block test. The hamsters were placed with the forelimbs on a block (6 cm high) and the descent latency, i.e., the time (in seconds) during which the hamsters maintained this position, was noted. Other side-effects were noted during the 3 h observation period, but not quantified.

Riluzole, kindly provided from Rhone-Poulenc Rorer (Cologne), was freshly dissolved in a solution of distilled water and 0.1 M HCl (pH 3.0) prior to each experiment. The injection volume was 5 ml/kg. For control recordings, the hamsters received the same volume of vehicle (acidified water, pH 3.0). Route of administration of drug and vehicle was i.p. in all experiments.

2.3. EEG recordings

For EEG recordings from depth electrodes in the red nucleus 2 groups of 6–7 mutant hamsters and 2 groups of 5–7 non-dystonic control hamsters of an outbred line were used. Unipolar recording electrodes (teflon-coated stainless steel; 0.2 mm diameter) were chronically implanted in anaesthetized hamsters (pentobarbital 60 mg/kg) at an age of 30–35 days. In each animal, one electrode was implanted into the red nucleus of the right hemisphere according to the following coordinates (relative to bregma): AP −3.2, L 1.0, V 5.5. A grounding screw electrode was implanted over the left parietal cortex.

The EEG recordings were carried out in freely moving dystonic and non-dystonic hamsters at the age of 35–40 days. In one group of mutant hamsters and one group of control hamsters, EEG recordings were done under basal conditions, i.e., without treatment with riluzole. In a second group of mutant and control hamsters, EEG recordings were undertaken after administration of 10 mg/kg riluzole. Simultaneously with the EEG recordings the hamsters were continuously observed and the severity of dystonia and the latencies to the different stages were noted as described above. Per min, 4 periods of 5 s were recorded for 1–3 h. In both groups of mutant hamsters, EEG recordings were done during stage 0 (basal) or during stage 0–2 (after treatment with riluzole) and during stage 6.

The BrainWave System was used (BrainWave, Broomfield, CO) for EEG recordings and analyses of data by fast Fourier Transformation. The recordings were amplified (10^4 fold; bandpass filtered 1–4000 Hz; 48 dB/octave) and digitized at a sampling rate of 1633 Hz per electrode in 4 periods of 4 s per min. The total power included frequencies between 1.25 and 42 Hz with a spectral resolution of 0.2 Hz.

After EEG recordings the animals were killed in order to verify the correct placement of the electrodes. The hamsters were deeply anaesthetized with pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% phosphate-buffered formaldehyde. Coronal sections were Nissl-stained and the positions of the recording electrodes were determined according to the stereotaxic atlas of the golden hamster (Knigge and Joseph, 1968).

2.4. Statistics

The significance of differences in severity of dystonia, in latency to onset of dystonia or to maximum severity between control trials (pre- and post-drug) and drug trial and descent latencies (catalepsy) between pre-drug control and after treatment was calculated with the Wilcoxon signed rank test of paired replicates.

The total power of the EEG recordings of each mutant and control animal was determined as mean of the initial 10 min recording period. Furthermore, in mutant hamsters the total power of the initial 10 min of stage 6 without or after treatment with riluzole was calculated. The results for the different groups of control and dystonic hamsters were calculated as means $\pm$ S.E. Since riluzole (10 mg/kg) decreased the latency to onset of dystonia in mutant hamsters (see Section 3), during the initial 10 min recording period the treated mutant hamsters had reached moderate dystonia (stage 2). The significance of differences in total power between untreated control and mutant hamsters or after administration of riluzole, respectively, was calculated with the Mann-Whitney *U*-test. The Mann-Whitney *U*-test was also used for comparisons between untreated

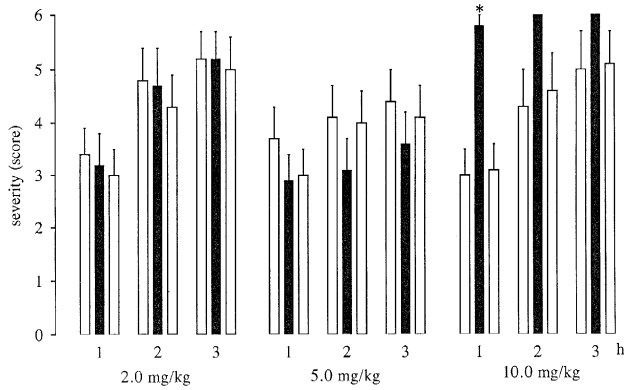

Fig. 1. Effect of riluzole on dystonic movements in dt^{sz} mutant hamsters at age of maximum severity (31–36 days; max-period). The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd h after i.p. administration of 2.0, 5.0 or 10.0 mg/kg riluzole. Control recordings were made two days before (pre-drug control) and two days after (post-drug control) the drug trial. Asterisk indicates significant increase in severity in comparison to the pre-drug and post-drug control (* $P < 0.01$). Data are shown as means + S.E. of 9 (2.0 and 5.0 mg/kg) or 8 (10.0 mg/kg) dystonic hamsters. Absence of S.E. bars indicates that all hamsters had reached the same severity. Open bars: pre- and post-treatment control scores; black bars: scores during treatment.

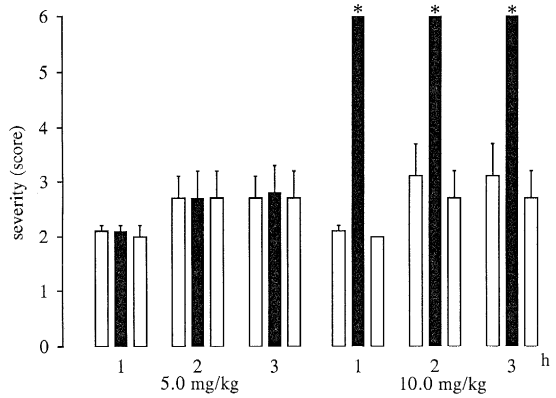

Fig. 2. Effect of riluzole on dystonic movements in dt^{sz} mutant hamsters at age 54–57 days, at which the severity of dystonia is decreased (post-max period). The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd h after i.p. administration of 5.0 or 10.0 mg/kg riluzole. Control recordings were taken two days before (pre-drug control) and two days after (post-drug control) the drug trial. Asterisks indicate significant increase of severity in comparison to the pre-drug and post-drug controls (* $P < 0.01$). Data are shown as means + S.E. of 9 (5.0 mg/kg) or 11 (10.0 mg/kg) dystonic hamsters. For further explanations, see the legend to Fig. 1.

and treated groups of mutant hamsters or control animals, respectively.

3. Results

In dt^{sz} mutant hamsters at the age of maximum severity of dystonia (max period), riluzole exerted significant prodystonic effects at a dose of 5 mg/kg (Table 1) and 10 mg/kg (Fig. 1 and Table 1). Riluzole dose dependently decreased the latency to onset of dystonia (Table 1). After administration of 10 mg/kg, the severity of dystonic attacks increased and the individual maximum stage was reached more rapidly (Fig. 1). All treated hamsters showed stage 6 after 19.2 ± 4.8 min (10–40 min) while only 6 (out of 8) of these hamsters reached stage 6 after 91.5 ± 13.9 min (42–151 min) in the pre-drug control ($P < 0.05$). Severe dystonic disturbances, i.e., stage 6, lasted more than 4 h. In age-matched control hamsters of the inbred or

Table 1
Effect of riluzole on latency to onset of dystonic attacks in mutant dystonic hamsters

Dose (mg/kg)	Age (days) at drug trial	Latency (min)			*n*
		Pre-drug trial	Drug trial	Post-drug trial	
Max period					
2.0	36	10.7 ± 0.7	12.1 ± 0.7	10.2 ± 1.0	9
5.0	35	11.4 ± 1.4 **	6.4 ± 0.9	13.7 ± 1.2 **	9
10.0	31	9.3 ± 1.2 **	3.9 ± 0.7	11.1 ± 0.7 **	8
20.0	37	9.3 ± 1.3 **	3.1 ± 0.4 [b]	4.6 ± 0.4 *	7
Postmax period					
5.0	57	13.3 ± 1.7 *	10.3 ± 1.1	18.0 ± 1.5 *	9
10.0	54	11.0 ± 1.8 **	3.1 ± 0.3	14.8 ± 1.0 **	11
After remission					
10.0	71	— [a]	9.3 ± 1.5 [b]	— [a]	6
20.0	75	— [a]	3.8 ± 0.7 [b]	— [a]	6

Latency was determined as the time (min) to the first unequivocal signs of the dystonic attacks (stage 2). Data are shown as means ± S.E. of the number of hamsters indicated. Significance of difference between control and drug trials are marked by * ($P < 0.05$) or ** ($P < 0.01$).
[a] No dystonic attacks can be induced by 'triple stimulation' in dt^{sz} hamsters at this age.
[b] Latency to onset = latency to maximum stage 6.

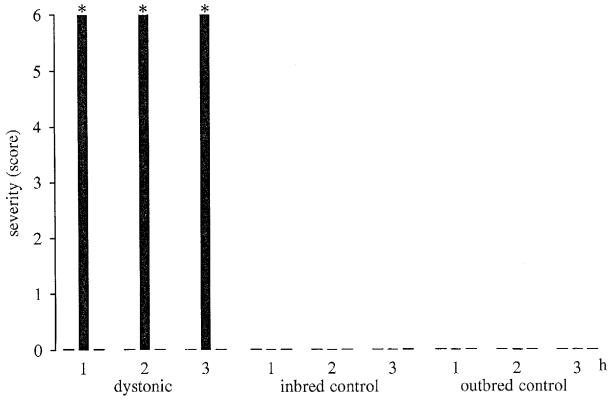

Fig. 3. Effect of riluzole at a dose of 10 mg/kg on dystonia in mutant hamsters at age > 70 days, i.e. after complete remission of dystonia, and in age-matched non-dystonic control hamsters of a related inbred and a non-related outbred line. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd h after i.p. administration of 10.0 mg/kg riluzole. Control recordings were made two days before (pre-drug control) and two days after (post-drug control) the drug trial. Asterisks indicate significant increase of severity in comparison to the pre-drug and post-drug controls (* $P < 0.01$). Data are shown as means + S.E. of 6 (mutant hamsters), 6 (control hamsters of the inbred line) or 7 (control hamsters of the outbred line) animals. In control hamsters, riluzole at a dose of 10 mg/kg did not induce dystonic disturbances (absence of bars). For further explanations, see the legend to Fig. 1.

outbred line, no dystonic movements were induced by 10 mg/kg riluzole (not illustrated). Adverse effects were not provoked at the dose of 2 mg/kg, which did not cause prodystonic effects, but at the higher dose of 5 mg/kg moderate ataxia and sedation were observed in mutant hamsters and control animals. These side effects were more marked at a dose of 10 mg/kg. In both dt^{sz} and control hamsters, the adverse effects lasted for about 1 h after administration. Riluzole exerted no cataleptic effects in either dystonic hamsters or control animals (not illustrated). Although the immobilization during stage 6 in dt^{sz} hamsters does not allow forward locomotion, the animals were able to descend from the block within a few seconds.

As shown in Fig. 2, the prodystonic effect of riluzole became more evident in older hamsters, i.e., animals which had already passed the age of maximum severity (post-max-period). At a dose of 10 mg/kg, riluzole markedly aggravated the dystonic attacks in mutant hamsters over the whole 3 h observation period, demonstrating that riluzole not only accelerates the progression of dystonia as shown during the max-period. Stage 6 was reached in all hamsters ($n = 11$) within 5–35 min after drug administration whereas only three of the animals reached stage 6 within 84–87 min in the pre-drug control. As in the max-period, 5 mg/kg did not increase the severity of dystonia, but both doses, 5 and 10 mg, significantly reduced the latency to onset of dystonia (Table 1). Adverse effects were the same as those described above for hamsters in the max-period.

The marked prodystonic effects prompted us to examine the effects of 10 mg/kg riluzole in a further group of dt^{sz} mutant hamsters older than 70 days, i.e., hamsters which had completely lost their susceptibility to induction of dystonia by the triple stimulation procedure. As shown in Fig. 3, riluzole provoked severe dystonia. Stage 6, reached within 9.3 ± 1.5 min, lasted longer than 4 h. Such prodystonic effects could not be obtained in age-matched control

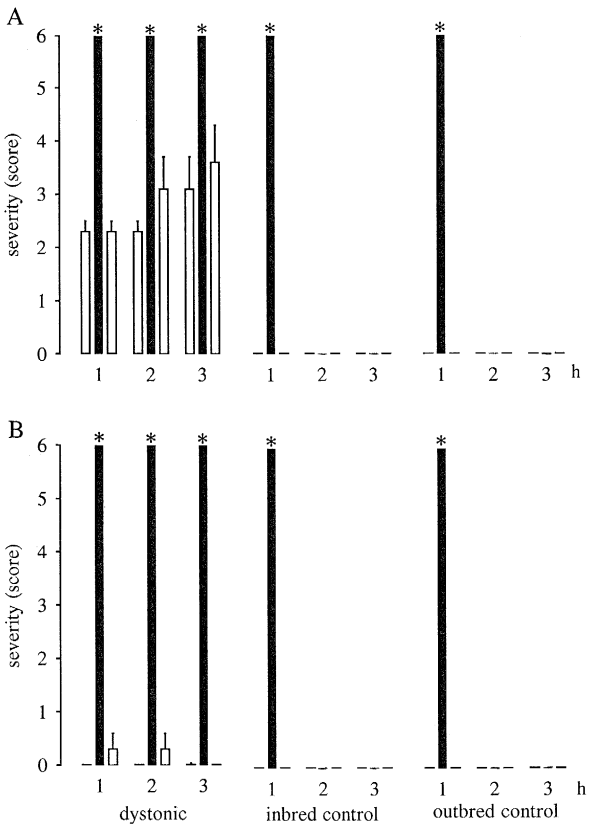

Fig. 4. Effect of riluzole at a dose of 20 mg/kg on dystonia in mutant dystonic hamsters at (A) age 37 days (i.e. max-period; animals selected with low severity of dystonia) and age-matched non-dystonic control hamsters of a related inbred line and a non-related outbred line and (B) in mutant dystonic hamsters at age > 70 days, i.e., after complete remission of dystonia, and in age-matched non-dystonic control hamsters of the inbred and outbred line. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd h after i.p. administration of 20.0 mg/kg riluzole. Control recordings were taken two days before (pre-drug control) and two days after (post-drug control) the drug trial. Asterisks indicate significant increase of severity in comparison to the pre-drug and post-drug controls (* $P < 0.01$). Data are shown as means + S.E. of 6–7 (mutant hamsters), 5–6 (control hamsters of the inbred line) or 7–8 (control hamsters of the outbred line) animals. At a dose of 20 mg/kg, riluzole provoked dystonic disturbances in all mutant hamsters and control animals. In control hamsters dystonic reactions were observed during the 1st h after administration, but not during the 2nd or 3rd h (absence of bars). For further explanations, see the legend to Fig. 1.

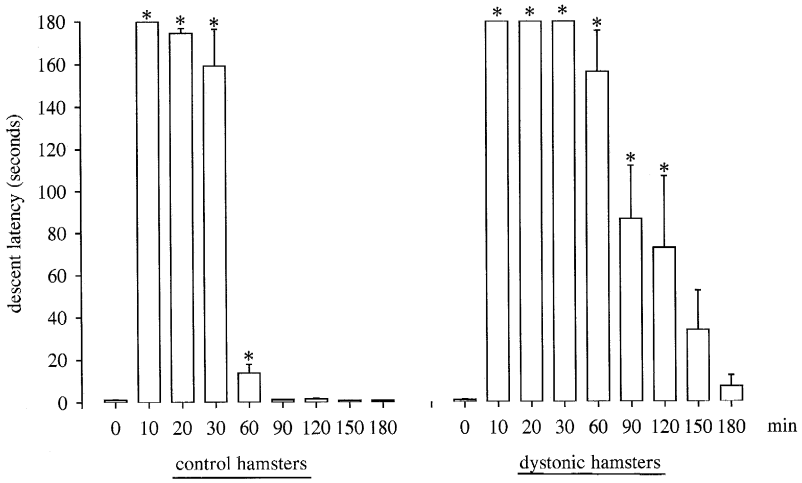

Fig. 5. Cataleptic effects of riluzole (20.0 mg/kg) in dystonic hamsters (black bars) and control hamsters of an inbred line (open bars) determined by quantifying descent latency in a block test. Descent latency was defined as the time in seconds during which the hamster maintained its position after its forelimbs were placed on a block 6 cm high. The data are shown as means + S.E. of 6 animals per group at different times (min) after administration of riluzole. Asterisks indicate significant increase of descent latency compared to pre-drug control within each group (* $P < 0.05$).

hamsters of the inbred or outbred line. The side-effects of riluzole were similar to those described for younger animals.

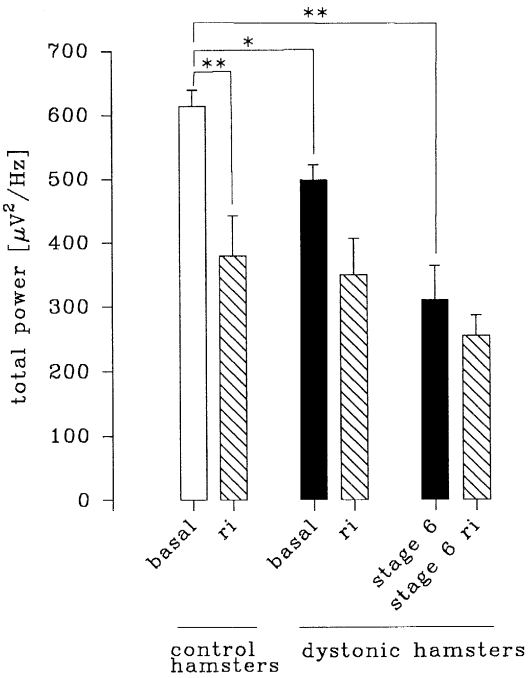

Fig. 6. Total power as analyzed from power spectra of electrographic recordings from red nucleus in mutant dystonic hamsters and age-matched non-dystonic control hamsters under basal conditions, i.e. in the absence of motor disturbances and without treatment with riluzole (black bars, dystonic hamsters; open bars, control hamsters) and after treatment with riluzole (ri; 10.0 mg/kg i.p.; hatched bars). In addition, the figure shows the total power in mutant hamsters after the maximum severity of dystonia without pretreatment with riluzole was reached (stage 6) and after administration of 10 mg/kg (stage 6 ri). Data are shown as means + S.E. of 10 min recording period of 7 control and dystonic hamsters (basal), of 5 control hamsters and 6 mutant hamsters (ri) and 6 mutant hamsters, which had reached stage 6. Significant differences are indicated by asterisks (* $P < 0.05$; ** $P < 0.01$).

In order to examine whether the mutant hamsters are only more susceptible to the prodystonic activity of riluzole than control hamsters, a very high dose of 20 mg/kg riluzole was administered to both mutant and control animals. As shown in Fig. 4, this dose provoked dystonic disturbances not only in mutant hamsters but also in control animals, independently of age. Immediately after administration of this high dose, all hamsters showed loss of the righting reflex and increase of muscle tone followed by a cataleptic stage with a twisted, hunched posture and hind- and forelimbs tonically extended forward, i.e., signs which characterize stage 6 of dystonia in mutant hamsters. As determined by the block test, catalepsy, i.e., a significant increase of the descent latency, lasted 1 h in control hamsters, while in mutant hamsters the catalepsy lasted 2 h (Fig. 5). The control hamsters recovered from dystonic disturbances within 35–45 min, whereas dystonia (stage 6) lasted more than 5 h in all mutant hamsters.

EEG recordings demonstrated that the total power in the red nucleus was significantly decreased in mutant hamsters compared to non-dystonic control hamsters under basal conditions, i.e., in the absence of motor disturbances and without treatment with riluzole (Fig. 6). In dt^{sz} mutant hamsters, the total power was decreased more during stage 6 than stage 0. During the initial 10 min after administration of 10 mg/kg riluzole, the total power decreased significantly in control animals, whereas in mutant hamsters which exhibited moderate dystonia (stage 0–2) during the same recording period riluzole only tended to decrease the total power, i.e., the reduction was not significant. Thus, the total power reached comparable levels in control and mutant hamsters after administration of riluzole at a dose of 10 mg/kg, which exerted prodystonic effects in mutant hamsters but did not provoke dystonic disturbances in control animals (see above). Riluzole also tended to decrease the total power during stage 6 in mutant hamsters, but this effect was not significant.

4. Discussion

The present data demonstrated clearly that riluzole causes prodystonic effects in mutant hamsters at doses (5, 10 mg/kg) which were previously found to be anti-ischemic in gerbils (4–8 mg/kg i.p.; Malgouris et al., 1989; Pratt et al., 1992). Similar to recent results with lamotrigine (30 mg/kg i.p.; Richter et al., 1994), even in dt^{sz} hamsters older than 70 days, i.e., when the hamsters had lost their susceptibility to induction of dystonia by mild stress, riluzole (10 mg/kg) provoked long-lasting dystonic attacks. Thus, dystonia seems not to be truly transient in this animal model as described recently (Löscher et al., 1989, 1995), but the age-dependent time course of stress-induced dystonia may be a result of counteracting mechanisms subsequent to biochemical alterations (Khalifa and Iturrian, 1993). In non-dystonic control hamsters of a related inbred line and of a non-related outbred line, riluzole provoked short-lasting dystonic disturbances only at very high cataleptogenic doses of 20 mg/kg, indicating an increased risk of inducing dystonic reactions in predisposed individuals. These data are in line with recent findings with lamotrigine at a dose of 30 mg/kg in mutant hamsters (Richter et al., 1994).

With regard to antidystonic effects of glutamate receptor antagonists in genetically dystonic hamsters (Löscher and Richter, 1993; Richter et al., 1991, 1993) it seems to be unlikely that the prodystonic effects of riluzole and lamotrigine are based on their antiglutamatergic activity. The selective non-competitive NMDA receptor antagonist, dizocilpine, administered at very high doses (20 mg/kg) which caused increased muscle tone, catalepsy and convulsions, did not induce dystonic attacks in mutant hamsters or in control hamsters (unpublished observation). Riluzole and lamotrigine do not selectively inhibit the release of excitatory amino acids. Both compounds are known to block voltage-sensitive sodium channels (Leach et al., 1986; Lang et al., 1993; Hebert et al., 1994). This primary site of action may be essential for the prodystonic effects of riluzole and lamotrigine, because phenytoin and carbamazepine which inhibit voltage-dependent sodium channels (Lang et al., 1993) have also been found to aggravate dystonic disturbances in mutant hamsters (Fredow and Löscher, 1991). Furthermore, case reports indicate that antiepileptic drugs which block sodium channels, such as phenytoin, carbamazepine (Lang et al., 1993) or felbamate (Pisani et al., 1995), may cause choreoathetosis or acute dystonic reactions in humans (Corey and Koller, 1983; Lee, 1994; Kerrick et al., 1995). Interestingly, the generally well-tolerated novel anticonvulsant lamotrigine (Goa et al., 1993), may induce moderate to severe choreoathetosis in children at doses of 1.9–17.2 mg/kg/day (Frost et al., 1996) and worsened dyskinesia in patients with Parkinson's disease treated with levodopa (Zipp et al., 1993, 1995). Riluzole has been reported to lack extrapyramidal side-effects, at least in patients with amyotrophic lateral sclerosis or in schizophrenic patients treated with doses of 75–150 mg/day (Rabasseda et al., 1994). However, the present data on prodystonic effects of riluzole as well as recent findings with other sodium channel blockers in mutant hamsters and case reports for humans suggest that compounds which block voltage-gated sodium channels may generally carry the risk of causing acute dystonic reactions. As mentioned above, dizocilpine did not exert prodystonic effects at high doses, although there is evidence that this potent NMDA receptor antagonist also blocks voltage-gated sodium channels at high concentrations (Allaoua and Chicheportiche, 1989; Wamil and McLean, 1992). Thus, other mechanisms may contribute to the prodystonic effects of riluzole and lamotrigine in mutant hamsters.

Apart from its antiglutamatergic properties (at 10–100 μM), riluzole was found to decrease GABA release at a relatively low concentration (100 μM) in hippocampal slices from rats (Martin et al., 1993). Similarly, the potency of lamotrigine to inhibit the release of excitatory amino acids (ED_{50} 21 μM) is only twice that to inhibit GABA release (ED_{50} 44 μM) in slices of rat cerebral cortex (Leach et al., 1986). Since neurochemical and pharmacological studies have indicated that the GABAergic function is disturbed in mutant dystonic hamsters (Fredow and Löscher, 1991; Nobrega et al., 1995), the inhibition of GABA release may be critically involved in the prodystonic activity of riluzole and lamotrigine. However, as shown by Samuel et al. (1992), riluzole also inhibits the striatal uptake of GABA and glutamate. The striatal GABA and glutamate levels were not altered in rats 30 and 60 min after systemic treatment with riluzole at doses of 0.1 to 5.0 mg/kg (Samuel et al., 1992). This finding emphasizes the non-specific action of riluzole on striatal neuronal membranes, possibly resulting in neurotransmitter imbalances at higher doses which were found to exert prodystonic effects.

Furthermore, riluzole has been shown to reduce the striatal synaptosomal uptake of dopamine to a greater extent than GABA or glutamate after i.p. injection of 0.5–5.0 mg/kg in rats (Samuel et al., 1992). This effect of riluzole may be relevant to its prodystonic effect and to the higher potency in mutant hamsters than in control animals, because previous neurochemical examinations suggested that dopaminergic activity is increased in the dorsomedial striatum of mutant hamsters (Nobrega et al., 1996). In line with this explanation, antidopaminergic drugs exert antidystonic effects, while levodopa and apomorphine aggravate dystonia in mutant hamsters (Löscher and Fredow, 1992; Richter and Löscher, 1993). The cataleptogenic effect observed in the present study at prodystonic doses of riluzole, however, argues against overactivity of the dopaminergic system. Actually, there is evidence that riluzole also inhibits striatal dopamine release (Cheramy et al., 1986), which may explain that the levels of dopamine and metabolites were not affected in the rat striatum after i.p.

injection of 0.1–5.0 mg/kg riluzole (Samuel et al., 1992). To our knowledge, there are no data on the effects of higher doses of riluzole, i.e., 10 mg/kg, which increased the severity of dystonia in mutant hamsters, but the widespread effects of riluzole on various neurotransmitters, mediated at least in part by blockade of voltage-gated sodium channels, may lead, through biochemical imbalances, to dystonic reactions.

Several clinical observations in dystonia, such as co-contractions in voluntary movements and increased muscle tone, suggest that reduction of spinal cord and brainstem inhibition is an important mechanism in dystonia (Hallett, 1993). The prodystonic effects of riluzole may be related to disinhibition of the spinal cord via the red nucleus, as indicated by the present data. Moreover, inhibition of glycinergic transmission by riluzole, demonstrated in rat motoneurones (Umemiya and Berger, 1995), could be involved in its prodystonic activity. The present results confirm recent findings of an abnormal rubrospinal activity in mutant hamsters (Richter et al., 1995; Gernert et al., 1997) and support the supposition that the red nucleus may be involved in dystonia in humans (McGeer and McGeer, 1988). However, the data suggest that the decrease in total power in the red nucleus does not play a primary role in the dystonic syndrome, because 10 mg/kg riluzole, i.e., a dose which did not provoke dystonic disturbances in control hamsters, decreased the total power in control animals to the same extent as in mutant hamsters. Recent biochemical and electrophysiological experiments with dystonic hamsters showed dysfunctions in other regions, such as the striatum, thalamus and cerebellum (Nobrega et al., 1995, 1996; Richter et al., 1995; Gernert et al., 1996), which could contribute to imbalances between the different supraspinal command signals, and thus explain the increased susceptibility to prodystonic effects of riluzole in mutant hamsters.

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