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Short Communication

Riluzole, an Inhibitor of Glutamatergic Transmission, Suppresses Levodopa-Induced Rotations in 6-Hydroxydopamine-Lesioned Rats

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The unilateral 6-hydroxydopamine-lesioned rat model is popular for the study of Parkinson's disease. Administration of levodopa, the primary treatment in Parkinson's disease, to 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine)-lesioned rats causes contraversive circling. This may be due, in part, to overstimulation of supersensitive dopaminergic receptors in the lesioned hemisphere, by unstored dopamine formed from exogenous levodopa (Crossman 1990). Following nigrostriatal dopamine depletion, glutamatergic pathways within the striatum and its output nuclei become overactive (Baas 2000). Riluzole [2-amino-6-(trifluoromethoxy) benzothiazole; 6-trifluoromethoxy-1,3-benzothiazolde-2-ylamine] is a drug that selectively depresses potential-driven glutamate action over γ-aminobutyric acid (GABA) release, and at higher concentrations, also strongly potentiates postsynaptic $GABA_A$ responses (Prakriya & Mennerick 2000; He *et al*. 2002). Therefore, riluzole is currently used to treat amyotrophic lateral sclerosis patients in whom glutamate excitotoxicity is believed to play a role in motor neurone degeneration (Bryson *et al*. 1996). In several experimental models, riluzole was demonstrated to inactivate voltage-dependent sodium channels (Herbert *et al*. 1994; Taylor & Meldrum 1995; Anaki *et al*. 2000; Prakriya *et al*. 2000), although evidence for activation of the G-protein-dependent process has also been obtained (Doble *et al*. 1996). Riluzole protected dopaminergic cells in several experimental models for Parkinson's disease, induced by 6-hydroxydopamine in rats (Barneoud *et al*. 1996), and by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a metabolic poison with tropism toward dopaminergic cells, in mice (Boireau *et al*. 1994), and monkeys (Benazzouz *et al*. 1995; Bezard *et al*. 1998). In the present study, we investigated the effect of riluzole on levodopa and apomorphine-induced rotational behaviour in animals with already established unilateral 6-hydroxydopamine lesions.

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Brain lesions to the substantia nigra were made under supervision of the Animal Care Committee at the Rabin Medical Center. Male Sprague-Dawley rats weighing 250–300 g (Harlan, Israel) were housed in standard conditions, anesthetized with chloral hydrate, 350 mg/kg intraperitoneally, and secured in a stereotaxic frame (Stoelting, USA). 6-Hydroxydopamine hydrobromide (12 μg in 6 μl of saline with 0.01% ascorbate, Sigma, USA) was prepared fresh and in order to prevent autooxidation, kept on ice until injection. A special drill was used to place a single burr hole at the appropriate site with the following coordinates: posterior 4.8 mm, lateral 1.8 mm dorsoventral, 8.1 mm with respect to bregma and dura, based on the Stereotaxis Atlas (Paxinos & Watson 1986). 6-Hydroxydopamine (6 μl, 1 μl/min.) was injected using a Hamilton 10 μl syringe with a 26-gauge needle. At the completion of the injection, the needle was left in place for another 3 min. and then withdrawn at 1 mm/min. in order to prevent vacuum. The burr hole was cleaned and the skin was closed (Perese *et al*. 1989).

The effectiveness of the lesion was determined 14 days after surgery, by testing apomorphine-induced contralateral turning behaviour (0.25 mg/kg), a reliable index of dopamine depletion (Hudson *et al*. 1993). The contralateral turnings were measured visually in a round tool (40 cm diameter), and only rats demonstrating an average of at least six counterclockwise turns/min. over 30 min. were selected for further study. For all rats (n=6), a one-week washout period was interposed between each drug treatment. Comparison between the means in the rotational behavioural data after drug administration, was analyzed by paired, two-tail Student's t-test with $P<0.05$ considered as statistically significant.

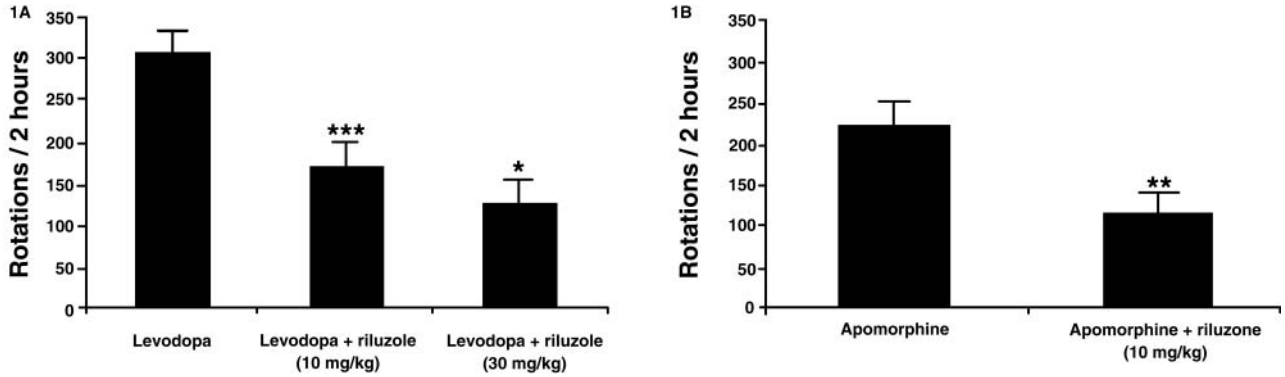

Fig. 1. Riluzole (10 mg/kg and 30 mg/kg, intraperitoneally) treatment after levodopa-induced (A) and apomorphine-induced (B) rotations in 6-hydroxydopamine lesioned rats. Measured in a round tool for 2 hr. *P<0.02, **P<0.01, ***P<0.005, Student's t-test.

We found that administration of levodopa/carbidopa (50/5 mg/kg respectively, intraperitoneally) elicited a high rate of contraversive rotations away from the 6-hydroxydopamine lesion site (301±45 rotations/for 2 hr), while administration of riluzole (10 mg/kg, intraperitoneally), 30 min. before levodopa/carbidopa, reduced rotations by 44% (169±21 rotations/for 2 hr, P<0.005). A higher dose of riluzole (30 mg/kg, intraperitoneally) mildly enhanced the inhibitory effect and reduced levodopa-induced rotation by 58% (127±38 rotations/2 hr; P<0.02) (fig. 1A). Pretreatment with riluzole (10 mg/kg, intraperitoneally) also reduced apomorphine-induced (0.25 mg/kg) rotations by 49% (112±37 versus 219±45 rotations/for 2 hr; P<0.01) (fig. 1B).

In contrast, pretreatment with the N-methyl-D-aspartate (NMDA) receptor antagonists amantadine (100 mg/kg, intraperitoneally) or the synthetic cannabinoid that lacks psychotropic properties (Eshhar *et al*. 1995), (+)- (3S, 4S)-7-hydroxy-Δ^6-tetrahydrocannabinol 1,1-dimethylheptyl (HU-211, 0.5 mg/kg, intraperitoneally), did not affect the rotation rate. In addition, pretreatment with the GABA agonist, gabapentin (40 mg/kg, intraperitoneally) or the voltage-sensitive sodium channels inhibitor, lamotrigine (12.5 mg/kg, intraperitoneally) did not affect the rotation rate (data not shown).

It is well known that following nigrostriatal dopamine depletion, glutamatergic pathways within the striatum and its output nuclei become overactive (Baas 2000). Therefore, in the present study we used riluzole, an inhibitor of glutamatergic release, and showed that it reduced levodopa- and apomorphine-induced rotations in rats with 6-hydroxydopamine lesions. The pharmacological properties of riluzole include an inhibitory effect on glutamate release and inactivation of voltage-dependent sodium channels. It also has the ability to interfere with intracellular events that follow neurotransmitter binding at excitatory amino acid receptors (Herbert *et al*. 1994; Doble 1996; He *et al*. 2002). However, in our study, pretreatment with amantadine or HU-211, NMDA receptor antagonists, or gabapentin, a GABA agonist, or the sodium channel inhibitor, lamotrigine, did not inhibit the rotational behaviour induced by levodopa or apomorphine. This suggests that the mechanism in which riluzole exerts its action in our model involves other glutamate receptors subtypes or G-protein-dependent processes or other mechanisms, such as interactions with other neurotransmitter systems, yet unknown. In our study, riluzole was given after the 6-hydroxydopamine lesion and thus its effect was symptomatic only and not neuroprotective as was shown by Barneoud *et al*. (1996). Riluzole, given 15 min. before and 24 hr after the 6-hydroxydopamine lesion in rats, was demonstrated to reduce both the apomorphine- and amphetamine-induced rotations, and attenuated the decreased dopaminergic metabolism, in the striatum and the substantia nigra (Barneoud *et al*. 1996).

As in patients with advanced Parkinson's disease, rats with massive (over 90%) unilateral destruction of the dopaminergic nigrostriatal projections, generated dopamine from exogenous levodopa in the striatum occurring predominantly in dopa-decarboxylase containing non-dopaminergic compartments (Hefti *et al*. 1980). Since these neuronal and non-neuronal sites unlike the dopaminergic nerve terminals, do not contain dopamine storage vesicles, the formed dopamine molecules are spilled out rapidly and stimulate the receptors in a non-physiologic pulsatile fashion (Melamed *et al*. 1980). In rats with unilateral 6-hydroxydopamine nigral lesions, contralateral rotations induced by levodopa may be analogous to the beneficial effect of this drug in Parkinson's disease patients. However, such rotations may also relate to levodopa-induced dyskinesia, which is the most common side effect of chronic treatment. Overactivity of glutamateric transmission was suggested to play a role in the pathogenesis of levodopa-induced dyskinesias (Baas 2000). Indeed, in a small non-controlled pilot study, we have recently shown that riluzole treatment (100 mg/day) decreased the hours spent in dyskinesias, without any deterioration of the signs and symptoms of Parkinson's disease, and with no significant side effects (Merims *et al*. 1999). In conclusion, our findings suggest that treatment with riluzole might be beneficial for levodopa-treated patients with Parkinson's disease through attenuation of the glutamatergic overactivity.

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