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Riluzole prevents MPTP-induced parkinsonism in the rhesus monkey: a pilot study

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Abstract

Previous studies have shown that riluzole (2-amino-6-trifluoromethoxy-benzothiazole), a drug which interferes with glutamate neurotransmission, has a neuroprotective action in rodent models of global and focal cerebral ischemia. In this pilot study, the protective and palliative effects of riluzole have been examined using an animal model of Parkinson's disease. Two monkeys were rendered hemiparkinsonian by one intracarotid injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and motor signs were evaluated using clinical examination and electromyographic recordings. When riluzole (4 mg/kg) was administered before the injection of MPTP, parkinsonian motor symptoms, in particular bradykinesia and rigidity, were absent. When injected daily in one monkey which presented stable motor symptoms, bradykinesia and rigidity were significantly reduced. Riluzole pretreatment induced a persistent increase in dopamine turnover when compared to MPTP alone. Thus, a possible neuroprotection and a facilitation of dopamine release may explain the behavioural effects reported with riluzole treatment. These preliminary results suggest that riluzole could possess neuroprotective and palliative effects in a primate model of Parkinson's disease.

Keywords: Riluzole; Neuroprotection; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Bradykinesia; Rigidity; Dopamine release; Parkinson's disease; (Rhesus monkey)

1. Introduction

The motor symptoms of Parkinson's disease are associated with the degeneration of dopaminergic neurones in the substantia nigra pars compacta. These conditions can be reproduced in monkeys (Langston et al., 1984; Doudet et al., 1990; Benazzouz et al., 1992) by the injection of a neurotoxin: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The active metabolite of this toxin, 1-methyl-4-phenylpyridinium (MPP^+), provokes the selective destruction of dopaminergic neurones in the substantia nigra pars compacta, as well as the depletion of dopamine in the striatum (Burns et al., 1983; Langston et al., 1984; Mitchell et al., 1985). Recent studies in rodents (Tabatabaei et al., 1992; Turski et al., 1991; Brouillet and Beal, 1993) have suggested that excitatory amino acid antagonists may exhibit a neuroprotective effect against MPTP toxicity. This proposal has been challenged by some authors who have been unable to confirm the protective effect of glutamate antagonists on MPTP-induced toxicity in rodents and monkeys. Using MK-801, a non-competitive antagonist of the NMDA glutamate receptor, in mice, Sonsalla et al. (1992) and Kupsh et al. (1992) could not support the hypothesis that *N*-methyl-D-aspartate (NMDA) receptor antagonists possess neuroprotective effects against MPTP toxicity. In monkeys, the systemic injection of MK-801, prevents the development of parkinsonian motor symptoms induced by MPTP, and protects the dopaminergic neurones in the substantia nigra pars compacta (Zuddas et al., 1992). However, no clear evidence for an efficacy of either NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor antagonists was observed when stable parkinsonism was achieved in MPTP-treated monkeys (Domino, 1994; Luquin et al., 1993).

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Recently several authors have demonstrated that the hyperactivity of the subthalamic nucleus glutamatergic neurones, in MPTP-treated monkeys, plays an important role in the genesis of parkinsonian motor symptoms. The destruction (Bergman et al., 1990; Aziz et al., 1991) or high frequency stimulation (Benazzouz et al., 1993) of the subthalamic nucleus, or the injection of NMDA receptor antagonists in the internal part of the globus pallidus (kynurenic acid or MK-801) (Graham et al., 1990; Brotchie et al., 1991) alleviate parkinsonian motor symptoms.

Riluzole, 2-amino-6-trifluoromethoxy-benzothiazole, is a compound that interferes with glutamatergic neurotransmission. It is a powerful anticonvulsant agent when tested in convulsions induced by electrical stimulation, by chemical agents, or in genetically predisposed animals (Mizoule et al., 1985). In particular, it counteracts the convulsions induced by glutamate or aspartate in rodents. Electrophysiological studies have also shown that riluzole, when administered to rats by either the i.v. or the i.p. route, blocks the increase in action potential discharge caused by applying NMDA, kainate or quisqualate by microiontophoresis (Girdlestone et al., 1989). Biochemical studies have clearly established that riluzole interacts with glutamatergic neurotransmission (Bénavidès et al., 1985). Riluzole inhibits the release of glutamate and aspartate in rat hippocampal slices (Martin et al., 1991), as well as the spontaneous release of glutamate in vivo in the caudate nucleus of the cat (Chéramy et al., 1992). Recently, using behavioural, electrocorticographic and histological approaches, riluzole was shown to have anti-ischaemic effects in models of global ischaemia in the gerbil and of focal ischaemia in the rat (Malgouris et al., 1989; Pratt et al., 1992). Furthermore, riluzole was shown to possess protective activity in two models of impaired dopaminergic activity with pro-parkinsonian neurotoxins in rodents (Boireau et al., 1994a,b).

It was interesting to study the effects of riluzole in the MPTP primate model with two different aims in mind. Firstly, to ascertain whether riluzole could modulate the toxicity of MPTP in monkeys by blocking the appearance of Parkinson-type motor symptoms. Secondly, to examine the effects of riluzole on motor disturbances in monkeys previously treated with MPTP, in which the symptoms of Parkinson's disease had stabilized. Finally, we have verified biochemically, the severity of dopamine depletion and a possible riluzole-induced improvement of biochemical parameters linked to the metabolism of dopamine, in the striatum.

Table 1
Monkey Hemiparkinsonism rating scale

Tremor	Dyskinesia
0 = none	3 = severe and persistent, large amplitude
1 = some episodes	2 = moderate and consistently present
2 = frequent episodes	1 = mild and intermittently present
3 = persistent	0 = absent
Bradykinesia	**Localisation (if present)**
0 = normal speed	0 = contralateral
1 = mild slowing of overall movements	1 = ipsilateral
2 = severe slowing of movements	2 = both sides
3 = akinesia (no movement)	
Posture	**Vomiting**
0 = normal rising position	0 = absent
1 = flexed posture (0° < F < 45°)	1 = present
2 = severly flexed posture (90° < F)	
3 = dystonic posture	
Rigidity *	**Overall level of activity**
0 = none	+2 = severe hyperkinesia; persistent excessive involuntary movement
1 = mild	+1 = moderate hyperkinesia; noticeable increase in involuntary movement
2 = moderate	0 = normal amount of movement
3 = severe	−1 = moderate hypokinesia; sparse movement
	−2 = severe hypokinesia
Arm movements (reaching for food outside of task) *	
3 = no movements	
2 = sometimes	
1 = frequent	
0 = no differences with normal side	

* Only controlateral side is quantified.

2. Materials and methods

Experiments were performed on two *Macaca mulatta* monkeys (Ril I and Ril II). Both monkeys received a single injection of MPTP (0.4 mg/kg) in the right internal carotid artery, according to the experimental protocol previously described (Benazzouz et al., 1992). The animals were anaesthetized with ketamine (Ketalar; Substantia, Courbevoie, France) (7 mg/kg i.m.) and the internal carotid artery was localized by arteriography. A dose of 0.4 mg/kg was prepared by dissolving cristalline MPTP in 1 ml ethanol, which was diluted in 30 ml saline and then infused into the carotid artery at the rate of 1.5 ml/min.

The monkey Ril I was injected with placebo (0.9% NaCl) 1 h before and 6 h after the injection of MPTP. From day 2 to 30, this monkey also received placebo. From day 31 to 45, this monkey received a single daily injection of riluzole at the dose of 4 mg/kg i.p.

The monkey Ril II received riluzole at the dose of 4 mg/kg i.p. 1 h before and 6 h after the injection of MPTP. On days 2 and 3, this monkey also received two daily injections of riluzole at 6-h intervals. From day 4 to 30, this monkey received a single daily injection of riluzole at the same dose.

Both monkeys were examined clinically and muscular rigidity was quantified by electromyography.

2.1. Clinical examination

Clinical examination was used to characterize the functional capabilities of unilateral MPTP-lesioned monkeys with or without riluzole treatment. The evaluation was based on the monkey Parkinsonism rating scale proposed by Kurlan et al. (1991) and adapted for hemiparkinsonian monkeys. Motor parkinsonian-like signs studied, as illustrated in Table 1, were tremor, bradykinesia, posture, rigidity, arm movements and eventual side effects.

2.2. Quantification of muscular rigidity

Muscular rigidity was studied using a protocol adapted from Meara and Cody (1989, 1992). Electromyographic activity (EMG) of flexor and extensor muscles, biceps and triceps brachii, were recorded in response to repetitive passive movements of the forearm (amplitude: 50°; velocity: 2 Hz) applied by the examiner. Monkeys were partially restrained in a primate chair. The arm under study was strapped into a horizontal manipulandum free to rotate around a vertical axis oriented through the monkey's elbow. The forearm position was identified by a linear coaxial potentiometer and the voltage variations were analyzed by a data acquisition and a transfer system connected to a Macintosh computer (Apple) equiped with data reception and analysis programs. EMG activity was recorded from both muscles using intramuscular electrodes made of teflon-insulated silver wire. EMG activity was amplified, integrated (time constant of 20 ms), filtered and visualized by means of an oscilloscope display, prior to storage in the computer. The peak amplitude (PA) and surface area (S) of the EMG burst activity were studied for biceps and triceps muscles and Student's t-test was used to objectivate a significant difference between mean values.

2.3. Neurochemical study

The monkeys were killed 2 months after MPTP administration. After decapitation, the brains were quickly removed and stored at −80°C. After thawing, caudate nuclei were dissected, homogenized in 40 volumes of $HClO_4$, 0.1 N containing EDTA 0.1 mM and centrifugated at 15 000 × g for 60 min. The contents of dopamine and homovanillic acid were determined by high-performance liquid chromatography with electrochemical detection (Hétier et al., 1988). Dopamine and homovanillic acid were separated on a nucleosil C18

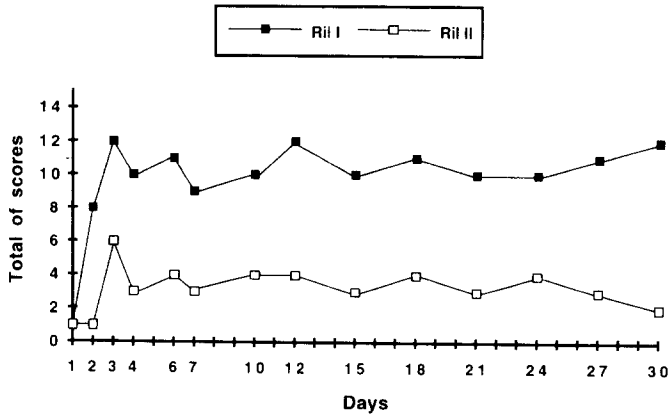

Fig. 1. Evolution of the sum of the scores of behavioral activity in both monkeys during the month of treatment with riluzole. Ril I: control animal treated with MPTP and NaCl 0.9%. Ril II: animal treated with riluzole 1 h before the injection of MPTP and during 30 days.

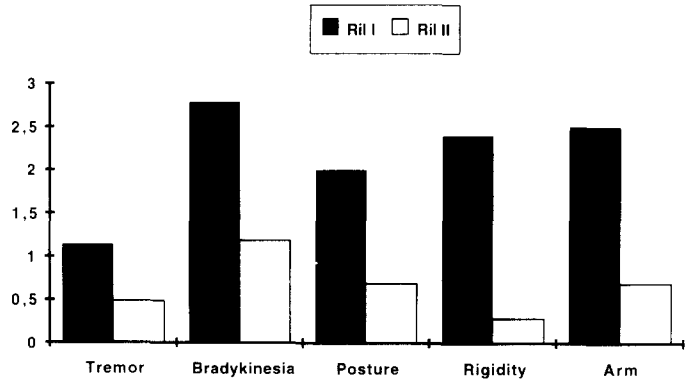

Fig. 2. Histogram showing the scores of five parameters studied out over 1 month after unilateral MPTP infusion in both monkeys. Ril I: contol animal treated with MPTP and NaCl 0.9%. Ril II: animal treated with riluzole 1 h before the injection of MPTP and during 30 days.

column, 150 × 4.6 mm. The mobile phase (NaH_2PO_4: 100 mM; $HClO_4$: 20 mM; NaCl: 1 mM; EDTA: 0.1 mM, 1-heptanesulfonic acid: 8.2 mM, containing methanol 7% v/v, pH 3.7) was delivered at the rate of 2 ml/min at 35°C; the detector potential was set at 0.8 V vs. Ag/AgCl.

3. Results

3.1. Clinical examination

For the longitudinal study the sum of scores of the five quantified behavioural parameters for each monkey were determined (Fig. 1) and the resulting profiles obtained for both monkeys were compared. The results show a clear difference between the time course of the scores of monkey Ril II, which received riluzole in a preventive treatment, and Ril I, the saline-treated animal.

To ascertain if certain parameters were more pertinent than others, the mean value of each quantified behaviour was calculated. The results show that there was a difference between monkeys Ril II versus Ril I for all parameters (Fig. 2).

3.2. Quantification of muscular rigidity

In monkey Ril I, the group of EMG recordings made on days 7, 15, 20 and 25 were first studied. An analysis of variance did not reveal any significant variation from one day to another, thus the values of each parameter were pooled. The comparison was performed systematically for each parameter between the results obtained from the contralateral side and those obtained from the ipsilateral side. The results are summarized in Table 2. Electromyographic recordings associated with passive movements showed a marked stretch-related increase in biceps discharge in the contralateral limb compared to the ipsilateral limb. The comparison of biceps EMG parameters showed an increase of peak amplitude (3.22 ± 0.62 vs. 1.49 ± 0.28 mV, $P < 0.001$) and surface area (32.63 ± 9.84 vs. 16.23 ± 7.61 mV.ms, $P < 0.001$). Concerning the triceps EMG activity, no clear changes were observed in the values of either parameter.

To study the palliative effect of riluzole, the monkey Ril I received daily injections of riluzole from day 31 to day 45, and three sessions of EMG recordings were made (days 33, 35 and 37). Fig. 3 shows an example of EMG activity recorded from biceps and triceps before and after riluzole treatment. This figure shows a dramatic reduction of biceps EMG activity in the arm contralateral to MPTP infusion. Compared to the values obtained before the treatment with riluzole, Table 2 shows that there was a significant diminution of peak amplitude (1.6 ± 0.3 vs. 3.22 ± 0.62 mV) and surface area (16.48 ± 4.23 vs. 32.63 ± 9.84 mV.ms) of biceps EMG activity during riluzole treatment. The values

Table 2
Mean values and standard deviations of electromyographic parameters corresponding to passive repeated flexion and extension movements of the forearm recorded in monkey Ril I from the arm ipsilateral (A) and the arm contralateral to MPTP injection before (B) and during treatment with riluzole (C)

Ril I	A	B	C
	Ipsilateral arm ($n = 43$)	Contralateral arm ($n = 58$)	Contralateral arm + riluzole ($n = 53$)
Biceps PA (mV)	1.49 ± 0.28	3.22 ± 0.62 [a]	1.60 ± 0.3 [b]
Biceps S (mV.ms)	16.23 ± 7.61	32.63 ± 9.84 [a]	16.48 ± 4.23 [b]
Triceps PA (mV)	1.39 ± 0.4	1.43 ± 0.78	1.07 ± 0.39 [b,c]
Triceps S (mV.ms)	12.54 ± 3.56	14.21 ± 7.04	9.81 ± 3.23 [b,c]

PA: peak amplitude; S: surface (see Materials and methods). Student's t-test: [a] indicates a significant difference between A and B; [b] indicates a significant difference between A and C; [c] indicates a significant difference between B and C.

obtained were not significantly different from those obtained from the biceps of the normal side.

The triceps EMG recordings show that during riluzole treatment the values of peak amplitude and surface area were significantly lower than those obtained from the same arm before treatment, and than those obtained from the control arm.

In monkey Ril II, EMG recordings obtained from the arm contralateral to MPTP infusion at days 7, 14, 21 and 27 were compared with those obtained from the control arm (ipsilateral to MPTP infusion). Fig. 4, which represents an example of EMG activity associated with repetitive passive movements, shows that there was no difference between the profiles of biceps and triceps EMG activities in the contralateral and the ipsilateral arms. As analysis of variance did not reveal any significant variation from one day to another, the values of each parameter were pooled. Student's t-test ($P < 0.05$) shows that there was no significant difference between the values of EMG peak amplitude and surface area obtained from the contralateral arm and those obtained from the control side for both muscles (Table 3). These results show that in the monkey treated with riluzole 1 h before and over 30 days after MPTP infusion, muscular rigidity was absent. To verify if parkinsonian symptoms appearing after riluzole treatment was stopped, an EMG recording was made at day 36 under the same conditions. Statistical analysis

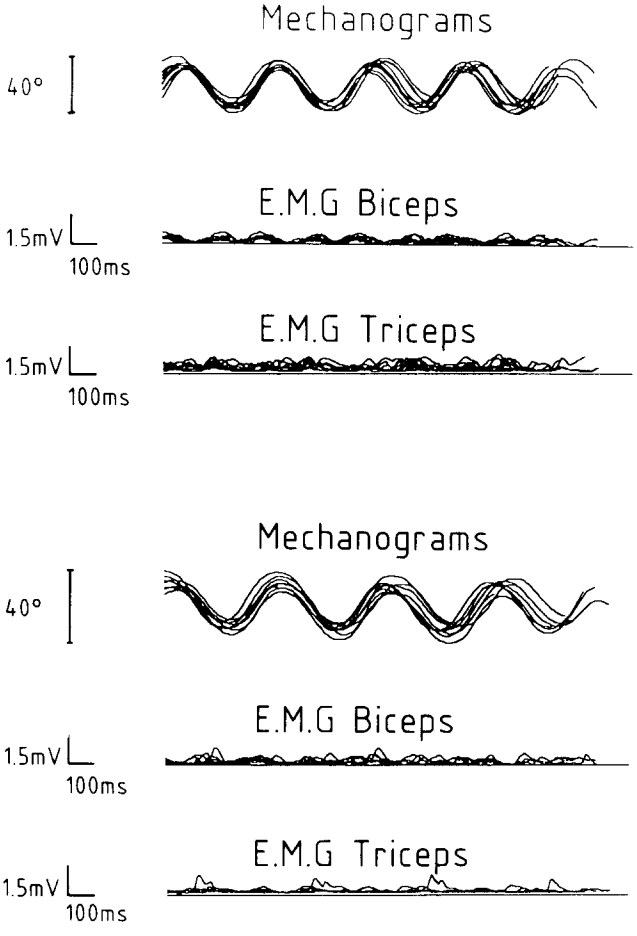

Fig. 3. Mechanograms and EMG activities of biceps and triceps muscles associated with passive repeated flexion and extension movements of the forearm in monkey Ril II treated with MPTP and riluzole. (A) Recordings obtained from the arm contralateral to the MPTP injection. (B) Recordings obtained from the arm ipsilateral to the MPTP injection.

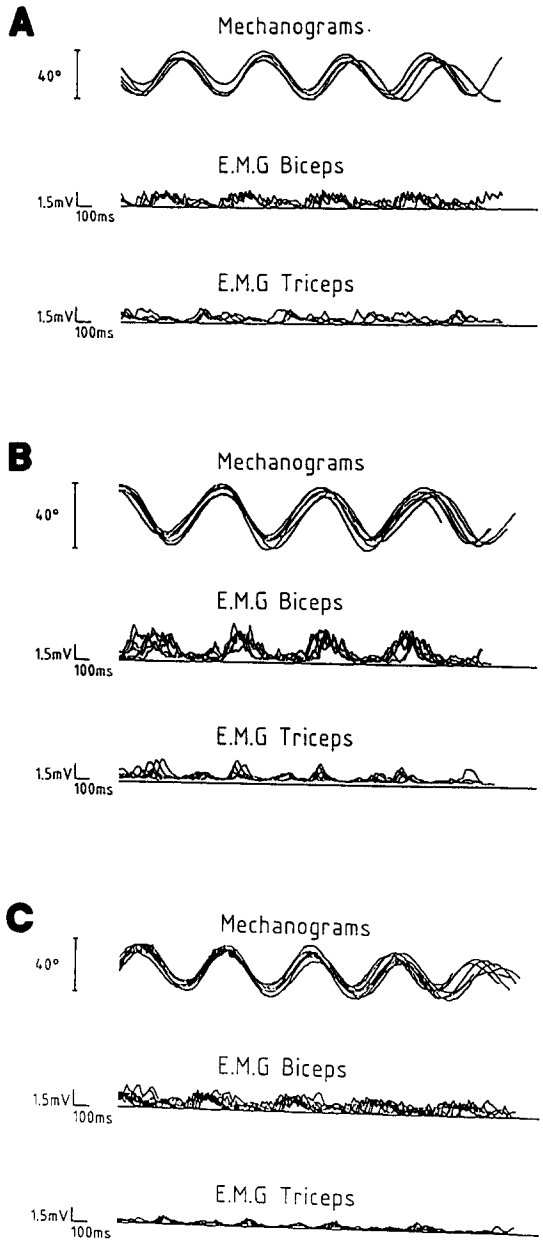

Fig. 4. Mechanograms and EMG activities of biceps and triceps muscles associated with passive repeated flexion and extension movements of the forearm in monkey Ril I treated with MPTP and NaCl 0.9%. (A) Recordings obtained from the arm ipsilateral to the MPTP injection. (B) Recordings obtained from the arm contralateral to the MPTP injection. (C) Recordings obtained from the arm contralateral to the MPTP injection after riluzole treatment.

Table 3
Mean values and standard deviation for electromyograph parameters corresponding to passive repeated movements of flexion and extension of the forearm recorded from monkey Ril II. (A) The side ipsilateral to MPTP injection; (B) the side contralateral to MPTP injection; (C) the same side as (B) after treatment with riluzole was stopped

Ril II	A	B	C
	Ipsilateral arm ($n = 49$)	Contralateral arm ($n = 43$)	Contralateral arm after day 30 ($n = 17$)
Biceps PA (mV)	0.75 ± 0.31	0.623 ± 0.32	0.72 ± 0.61
Biceps S (mV.ms)	7.44 ± 2.32	6.96 ± 3.3	96.85 ± 2.13
Triceps PA (mV)	1.00 ± 0.55	0.90 ± 0.39	1.24 ± 0.32 [b,c]
Triceps S (mV.ms)	11.59 ± 2.87	12.66 ± 4.3	13.02 ± 3.51

Student's t-test: [a] indicates a significant difference between A and B; [b] indicates a significant difference between A and C; [c] indicates a significant difference between B and C.

of the parameters studied did not reveal any significant difference between the values obtained at day 36 and those obtained at days 7, 14, 21 and 27, except for the maximal amplitude of the triceps recording (Table 3).

3.3. Biochemical studies

Values from the unlesioned side of monkey Ril I were assimilated to control data. Values from the lesioned side of the same monkey were used as an estimation of MPTP-induced dopamine degeneration, though a possible modification of dopamine metabolism due to the treatment with riluzole (from day 31 to 45) in this preliminary study cannot be ruled out. Dopamine levels decreased from 21 nmol/g to 0.8 nmol/g (96% decrease) and homovanillic acid levels from 58.3 to 4 nmol/g (93% decrease) after MPTP treatment, two results which are in good agreement with recent data from the literature (Chen et al., 1991). Injections with riluzole before and after MPTP injection (monkey Ril II) did not modify the decrease in dopamine levels induced by the neurotoxin (27–1.1 nmol/g), but did antagonize the decrease in homovanillic acid levels observed after MPTP treatment (77.5–34.2 nmol/g). The homovanillic acid/dopamine ratio is currently assimilated to an index of dopamine utilization or turnover. It varies from 2.7 (control side) to 4.9 after MPTP treatment, which represents an 80% increase in dopamine turnover in monkey I. Interestingly, the pretreatment with riluzole (monkey Ril II) increased more markedly dopamine use (homovanillic acid/dopamine ratio increased from 2.8 to 30.3), which represents a 1100% increase in this index (Fig. 5).

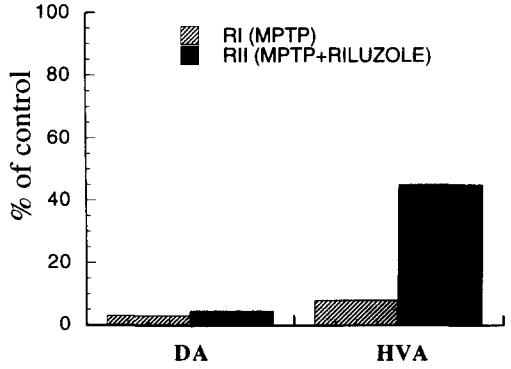

Fig. 5. Effect of riluzole on striatal dopamine and homovallinic acid levels in the hemiparkinsonian monkey.

4. Discussion

Although this pilot study is only based on single monkeys for each experimental paradigm and thus not suitable for statistical analysis, clinical and electromyographic results suggest that riluzole had a double effect on these MPTP-treated monkeys. Firstly, riluzole may have exerted a preventive effect when injected before the infusion of MPTP, since no parkinsonian motor impairment was observed. Secondly, riluzole may exert a palliative effect as the injection of riluzole in a monkey previously treated with MPTP-alleviated parkinsonian motor signs. It is noticeable that muscular tonus of the triceps is reduced after riluzole treatment (RIL I), and increased again after it was stopped (Ril II). Rigidity is predominantly seen in flexor muscle in parkinsonian patients (Delwaide et al., 1986). Thus, one possible explanation is that riluzole could have a relaxant effect on tonus regulation, not noticeable in normal animals (data not shown), but unmasked on the extensor muscles of the parkinsonian animal.

MPTP is converted by the action of the MAO-B (monoamine oxydase B-type) to 1-methyl-4-phenylpyridinium (MPP^+), which is the neurotoxic metabolite (Markey et al., 1984). MPP^+ is taken up via dopamine uptake (Javitch et al., 1985) and accumulates in dopaminergic terminals where it blocks cellular respiration (Nicklas et al., 1985). One possible protective effect of riluzole could be via MAO-B or dopamine uptake inhibition. This seems not to be the case, as riluzole does not inhibit MAO-B activity (Boireau et al., 1994), possesses a low affinity for dopamine uptake sites in vitro (Samuel et al., 1992), and does not inhibit the specific binding of [^{3}H]-WIN 35,428, a selective ligand of dopamine uptake sites, in mice in vivo. (Boireau et al., 1994b).

The membrane depolarization which results from the cascade of events that follows a decrease in ATP levels tends to reduce the Mg^{2+} blockade of NMDA channels and allows extracellular glutamate to become neurotoxic. Thus, in addition to the 'direct' MPP^+

neurotoxicity, an excitotoxic amplification is probable. Studies in rodents (Tabatabaei et al., 1992; Turski et al., 1991; Brouillet and Beal, 1993) have suggested that excitatory amino acid antagonists may exhibit a neuroprotective effect against MPTP toxicity, although this proposal has been challenged by some authors who have been unable to confirm these results (Sonsalla et al., 1992; Kupsh et al., 1992). The involvement of excitatory amino acids in the mechanism of MPTP toxicity has also been reported in monkeys: Zuddas et al. (1992) have shown that MK-801, a non-competitive antagonist of the NMDA receptor, is able to prevent MPTP-induced parkinsonism in primates. However, other authors have been unable to detect a palliative activity of glutamate antagonists (Domino, 1994; Luquin et al., 1993). These data prompted the testing of riluzole, an agent which interferes with glutamatergic neurotransmission in a less conventional fashion. Interestingly, in the MPTP primate model, Chiueh et al. (1990) have reported an increase in neuronal Ca^{2+} influx. Since riluzole is able to block NMDA-induced increase in Ca^{2+} entry in rat mesencephalic neurones in vitro (Hubert et al., 1994) this could explain, at least in part, the neuroprotective action of riluzole. Our own preliminary results favour a possible protective action of riluzole through a modulation of glutamate release.

However, riluzole may exerts its protective effect by other mechanisms. Recent results in mice indicate that some, but not all, voltage-dependent Na^+ channel blockers prevent dopamine depletion in the MPTP-mouse model (Jones-Humble et al., 1994).

The blockade of Na^+ entry may prevent the cascade of events that follows the neuronal depolarisation after ATP depletion. Furthermore, the neurotoxic effects of MPP^+ markedly increase the release of dopamine, by a mechanism which was proposed to be Na^+-dependent (Chiueh and Huang, 1991), though this has not always been observed (Westerink et al., 1987). Thus, inactivation of voltage-dependent Na^+ channels, as noted with riluzole (Benoit and Escande, 1991), might be involved in any protective effect.

Biochemical studies showed that MPTP treatment induced a decrease in dopamine (96%) and homovanillic acid (93%) very close to that recently reported by Chen et al. (1991). Injections with riluzole (monkey Ril II) did not modify the decrease in dopamine levels induced by the neurotoxin, but attenuated the decrease in homovanillic acid levels observed after MPTP treatment. The homovanillic acid/dopamine ratio (assimilated to an index of dopamine utilization or turnover) was markedly enhanced by riluzole. Such an increase may be due to either (or both): an increase in dopamine turnover in the remaining neurones, or a larger population of dopaminergic neurones spared as a consequence of riluzole-induced neuroprotection. This could explain why no modification of the level of dopamine was observed: the treatment with riluzole induced a persistent increase in dopamine metabolism at the expense of endogenous dopamine. Moreover, an increase in homovanillic acid levels strongly suggests that the release of dopamine itself was markedly increased. A persistent increase in dopamine release in the riluzole-treated monkey might explain the protective action of our compound in the behavioural tests reported in this study. It has been estimated that the parkinsonian syndrome appears only after lesion of around 50 or 60% of the dopaminergic cells (Agid et al., 1987a,b). Interestingly, the decrease in homovanillic acid levels observed in the riluzole-treated monkey was of 55% (vs. contralateral side). In consequence, although very preliminary, this result suggests that such a decrease in dopamine release involves minor behavioural changes. Thus, the partial recovery of homovanillic acid levels (probably due to an increase in dopamine release in the riluzole-treated monkey) may have restored a level of function sufficient to efface the effect of MPTP on behavioural parameters. These results complement previous biochemical data obtained in rodents in which riluzole has been shown to antagonize partially the decrease in dopamine levels induced by MPTP in mice (Boireau et al., 1994b), while in rats, the MPP^+-induced increase in dopamine release in the striatum in vivo was blocked (Boireau et al., 1994a). All these data suggest a biochemical efficacy for riluzole in several different animal models of MPTP-induced Parkinsonism.

Concerning the palliative effect of riluzole, treatment of a monkey with stabilized hemiparkinsonism greatly improved motor signs including bradykinesia and muscular rigidity. As the glutamatergic neurones of the subthalamic nucleus are hyperactive in the monkey treated with MPTP, it would be interesting to test riluzole for a possible action at this level. In conclusion, riluzole confers both a protective effect on dopaminergic neurones, as well as a palliative effect. Although these data are obtained in only two monkeys, and the immunohistochemical confirmation of these results remains necessary, our study opens up encouraging perspectives which merit rapid testing on a larger number of animals.

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