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ORIGINAL INVESTIGATION

Local administration of sarizotan into the subthalamic nucleus attenuates levodopa-induced dyskinesias in 6-OHDA-lesioned rats

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Abstract
Rationale Dyskinesia affects the majority of levodopa-treated parkinsonian patients within 5–10 years of treatment with levodopa. Clinical and preclinical observations suggest that an increase in serotoninergic transmission can contribute to the appearance of dyskinesias. It is thus conceivable that a modulation of synaptic dopamine (DA) levels induced by the inhibition of serotonin (5-HT) release, as a consequence of 5-HT_{1A} agonists administration, might alleviate dyskinesias.
Objective Since 5-HT_{1A} receptors are expressed in the subthalamic nucleus (STN), the aim of the present study was to assess the effect of the intrasubthalamic administration of sarizotan, a compound with full 5-HT_{1A} agonist properties, on levodopa-induced dyskinesias in the 6-hydroxydopamine (6-OHDA) model of parkinsonism.
Materials and methods Male Sprague–Dawley rats received a unilateral 6-OHDA administration in the nigrostriatal pathway. A test of apomorphine was performed to evaluate dopamine depletion. One week later, a cannula was implanted in the STN. Animals were treated with levodopa (6 mg/kg, i.p., twice at day) for 22 consecutive days. On day 23, several doses (1 ng, 10 ng, or 1 μg) of sarizotan were administered through the cannula to the STN. The higher doses of sarizotan effectively attenuated all levodopa-induced dyskinesias including axial, limb, and orolingual subtypes.
Conclusions These results suggest that the STN is a target structure for the antidyskinetic action of sarizotan and indicate that drug-mediated modulation of STN activity may be an alternative option for the treatment of levodopa-induced dyskinesias in Parkinson's disease.

Keywords Parkinson's disease · Dopamine · Levodopa · Dyskinesia · Subthalamic nucleus · Serotonin · Sarizotan

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Introduction

Long-term therapy with levodopa is associated with motor complications such as motor fluctuations and dyskinesias (Obeso et al. 2000; Schrag and Quinn 2000). Dyskinesia affects the majority of levodopa-treated parkinsonian patients within 5–10 years of treatment (Bezard et al. 2001; Rascol et al. 2000) and is often the key complication that limits further increases in dopaminergic therapy (Jankovic 2005). Because of the prevalence of dyskinesia and the limited options for pharmacological effective treatment (Goetz et al. 2005), developing new safe and effective therapies that treat dyskinesias without aggravating parkinsonism is widely accepted as an unmet need in Parkinson's disease (PD; Goetz et al. 2005).

Serotoninergic system dysfunction has long been recognized to occur in the basal ganglia of patients with PD (Hornykiewicz 1975; Kish et al. 2008). It has been suggested that serotonin (5-HT) neurons, originated in the dorsal raphe nucleus, innervating the striatum might be implicated in the antiparkinsonian action of levodopa by converting exogenous dopa to dopamine (DA) and subsequently releasing DA from 5-HT neurons as a false neurotransmitter (Arai et al. 1994; Maeda et al. 2005; Ng et al. 1970, 1971; Santiago et al. 1998; Tanaka et al. 1999). However, clinical and preclinical observations suggest that an increase in serotoninergic transmission can contribute to the appearance of dyskinesias (Carlsson et al. 2007; Carta et al. 2007; Kish et al. 2008). New findings suggest that DA may be released by serotoninergic neurons in a non-physiological manner leading to excessive swings of DA release and promote levodopa-induced dyskinesia (Carta et al. 2007).

The action of 5-HT is mediated by a variety of 5-HT receptors, including G-protein-coupled subtypes (5-HT_1, 5-HT_2, 5-HT_{4-7}) and a ligand-gated ion channel (5-HT_3; Barnes and Sharp 1999; Hoyer et al. 2002). Lately, there has been a growing focus of interest on 5-HT_{1A} receptor subtypes and their involvement in motor control. 5-HT_{1A} receptors are expressed presynaptically on serotoninergic neurons where they regulate transmitter release (Blier et al. 1998). It is well established that stimulation of presynaptic 5-HT_{1A} receptors diminishes nerve impulse activity in serotoninergic neurons leading to a decrease of serotonin release in the striatum (Gobert et al. 1995; Kreiss and Lucki 1994). It is thus conceivable that inhibition of serotonin release, as a consequence of 5-HT_{1A} agonists administration, might alleviate dyskinesias (Ba et al. 2007; Bibbiani et al. 2001; Bishop et al. 2006; Carta et al. 2007; Eskow et al. 2007).

Sarizotan is a compound with full 5-HT_{1A} agonist properties (Bartoszyk et al. 2004; Bartoszyk and Kuzhikandathil 2005; Rabiner et al. 2002) with additional low nanomolar affinity for D2, D3, and D4 receptors (Bartoszyk et al. 2004; Kuzhikandathil and Bartoszyk 2006). In rodent and primate models, 5-HT_{1A} agonists have shown promising antidyskinetic effects (Bedard et al. 2006; Bibbiani et al. 2001; Dekundy et al. 2007; Dupre et al. 2008; Gerlach et al. 2006a, b; Lunblad et al. 2005). It has been suggested that the decreased serotonin release in the striatum is the main site of the antidyskinetic action of sarizotan (Bibbiani et al. 2001). Moreover, recent evidence also indicates that 5-HT_{1A} stimulation may also directly influence postsynaptic DA receptor-mediated behaviors that are raphe-independent, since it has been recently demonstrated that 5-HT_{1A} stimulation reduced abnormal involuntary movements (AIMs) induced by selective D1 or D2 DA agonists in the 6-OHDA model in rats (Dupre et al. 2007). However, the presence of 5-HT_{1A} in other basal ganglia nuclei, such as the subthalamic nucleus (STN), has been shown (Pompeiano et al. 1994), and thus, the possible action of sarizotan in the STN and its possible relevance in its antidyskinetic action needs to be taken into consideration.

The STN is innervated by serotonin fibers mainly originated from the neurons in the dorsal raphe nucleus (Lavoie and Parent 1990), and several subtypes of 5-HT receptors including 5-HT_{1A} are expressed in the STN (Pompeiano et al. 1994). A recent electrophysiological study shows that in STN neurons, 5-HT elicits two distinct effects on the same neurons, the first being an excitation which is mediated by 5-HT_{2C} and 5-HT_4 receptors and the second an inhibition which is mediated by 5-HT_{1A} receptors (Standford et al. 2005). The motoric effect of 5-HT_{1A} agonists on STN activity has not been studied yet.

In view of the relevance that the 5-HT_{1A} stimulation at STN level might contribute to the antidyskinetic effect obtained with the systemic administration of sarizotan, the aim of the present study was to assess the effect of the intrasubthalamic administration of sarizotan on levodopa-induced dyskinesias in the 6-OHDA model of parkinsonism in rats.

Materials and methods

6-Hydroxydopamine lesions

Male Sprague–Dawley rats weighing 220–240 g were housed on a 12-h light/dark cycle with free access to food and water. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were placed in a stereotaxic frame with the incisor bar positioned 4.5 mm below the interaural line. Each animal received a 6-OHDA injection (8 μg in 4 μl of saline with 0.02% ascorbate over 8 min) into the left medial forebrain bundle by means of a Harvard infusion pump. Stereotaxic injections were placed 4.0 mm anterior to the interaural line, 1.3 mm lateral to the midline, and 8.4 mm ventral to the surface of the skull according to the atlas of Paxinos and Watson (1986). The injection cannula was left in place for additional 2 min before slowly retracting it. All animal experiments have been carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Following a 3-week recovery period, rats exhibiting a vigorous rotational response (>100 total turns) to apomorphine (0.05 mg/kg, s.c.) were selected for further study. It has previously been demonstrated that rats meeting this criterion have a greater than 95% depletion of striatal dopamine (Papa et al. 1994).

Subthalamic cannula implantation

Three days after apomorphine test, rats were placed in a stereotaxic frame, and under sodium pentobarbital anesthesia (50 mg/kg, i.p.), an indwelling cannula was implanted 7 mm below skull level above the STN at the following coordinates: A: +4.9 mm; L: +2.5 mm relative to lambda. The cannula was held in place using dental resin and a skull screw. A capped dummy cannula was placed within the indwelling cannula at all times, except during microinjections. Staining for Cresyl violet was performed for verification of microinjection cannula placements.

Protocol of treatments

One week after cannula implantation, animals were treated daily for 22 consecutive days with levodopa (6 mg/kg with 15 mg/kg benserazide, i.p., twice a day). The dose of levodopa used in the present study has been described to induce a gradual development of AIMs over the course of 3 weeks of treatment (Cenci et al. 1998; Marin et al. 2006).

On day 23, animals with a cannula above the STN were randomly distributed in three groups that received a 0.3-μl injection of 1 ng (n=7), 10 ng (n=6), or 1 μg (n=6) of sarizotan over a 1-min period. Control group received 0.3 μl of vehicle (n=7). The injection cannula remained in situ for a further 2 min. The injections were administered via an injection cannula inserted to the guide cannula through the STN (V −8.4 mm from skull) with the injection cannula connected to a 10-μl syringe and mounted on a microinfusion pump. Subthalamic sarizotan or vehicle administration was started 10 min after levodopa administration.

Abnormal involuntary movements rating

Levodopa-induced AIMs were scored according to a rat dyskinesia scale (Winkler et al. 2002). Rats were placed individually in transparent plastic cages and observed every 20th min, from 20 min before to 240 min after the injection of levodopa (monitoring periods of 1 min). AIMs were evaluated by an experimenter blinded toward the identity of the animals.

Three subtypes of AIMs were classified according to their topographic distribution as: axial AIMs, i.e., dystonic posturing or choreiform twisting of the neck and upper body towards the side contralateral to the lesion; limb AIMs, i.e., abnormal purposeless movements of the forelimb contralateral to the lesion, and orolingual AIMs, i.e., empty jaw movements and tongue protrusion. Enhanced manifestation of otherwise normal behavior, such as rearing, sniffing, grooming, and gnawing, were not included in the rating (Winkler et al. 2002). Each of these three subtypes was scored on a severity scale from 0 to 4 to each of the three AIM subtypes listed above according to the proportion of time/monitoring period during which the AIM is present: 0 = absent; 1 = occasional, i.e., present during less than 50% of the observation time; 2 = frequent, i.e., present during more than 50% of the observation time; 3 = continuous but interrupted by strong sensory stimuli; and 4 = continuous, not interrupted by strong sensory stimuli.

Rotational screening

For the measurement of rotational behavior, rats were placed in circular cages and tethered to an automated rotometer. The number of complete (360°) turns made during each 5-min period was recorded by computer. Rats were allowed 15 min to habituate to the rotometer before the administration of drugs. Following a 3-week recovery period after 6-OHDA lesion, rats exhibiting a vigorous rotational response (>100 total turns) to apomorphine (0.05 mg/kg, s.c.) were selected for further study.

Tissue preparation

Animals were killed by a lethal dose of pentobarbital. Immediately after death, the brains were removed from the skull and then rapidly frozen in dry ice reduced to powder. Fourteen-micron sections in the frontal plane were cut at −20°C using a cryostat, thaw-mounted on gelatin-double-coated slides, and stored at −80°C until processing.

DAT immunohistochemistry

Striatal sections were thawed and dried at room temperature and fixed with acetone for 10 min at 4°C. The sections were rinsed in phosphate-buffered saline (PBS, pH 7.4) twice, 5 min each, and immersed in 0.3% hydrogen peroxide in PBS for 10 min to block the endogenous peroxidase. At this point, sections were rinsed again in PBS and incubated with horse serum with 0.1% Triton X-100 for 20 min. Sections were incubated overnight at 4°C with a mouse anti-dopamine transporter (DAT) monoclonal antibody at a dilution 1:500 in PBS. Sections were rinsed twice in PBS, 5 min each, and ImmunoPure Ultra-Sensitive ABC Peroxidase staining kit was used to carry out the ABC staining method. By so doing, sections were incubated with biotinylated horse anti-mouse Ig-G for 30 min, followed by two rinses in PBS, and then incubated with avidin–biotinylated peroxidase complex for 30 min more. Finally, sections were rinsed in PBS and incubated with 3-3′-diaminobenzidine and 0.01% hydrogen peroxide for

15 min. Slides were washed with PBS, dehydrated in ascending alcohol concentrations, cleared in xylene, and coverslipped in DPX-EXLI mounting medium.

Statistics

Data were analyzed by a Kruskal–Wallis analysis of variance followed by the Mann–Whitney rank-sum test. The level of statistical significance was set at $p<0.05$ for all analyses. Data are represented as mean±SEM.

Results

DAT immunohistochemistry

In corroboration with apomorphine testing, an absence of DAT immunoreactivity in the ipsilateral striatum was observed in all 6-OHDA-lesioned animals (Fig. 1).

Control of subthalamic cannula

The rats used in these studies were selected on the basis of histological examination of the subthalamic location of the cannulae by means of Cresyl violet staining (Fig. 2).

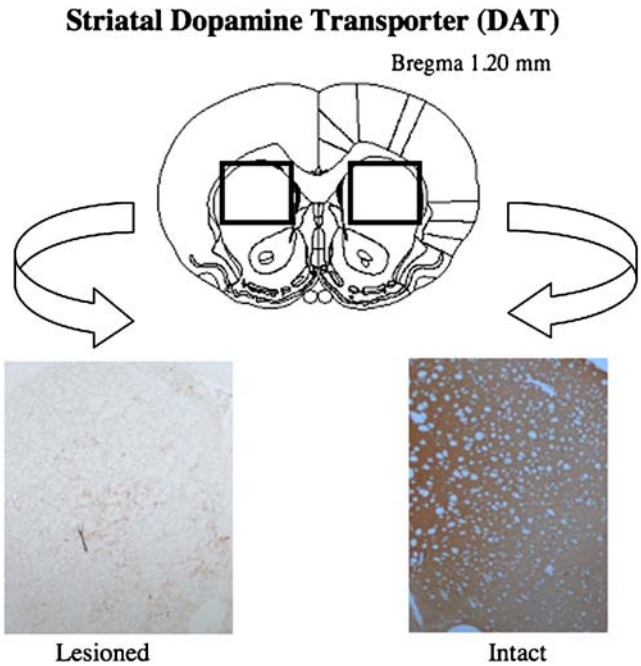

Fig. 1 Representative DAT immunohistochemistry from 14 μm coronal sections of the rostral striatum. Sections are from the lesioned and intact striatum of rats receiving 6-OHDA injection in the left forebrain bundle

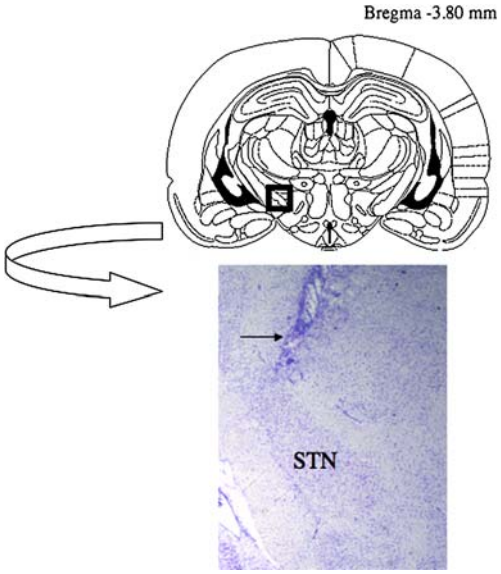

Fig. 2 Subthalamic cannula localization. Representative Cresyl violet staining from 14-μm subthalamic coronal sections

Effect of subthalamic sarizotan administration on levodopa-induced AIMs

Total dyskinesia score

Total score of levodopa-induced dyskinesia for each observation session was computed using the sum of all AIMs subtypes: axial, limb, and orolingual dyskinesias. Levodopa-induced dyskinesias were observed only on the side contralateral to 6-OHDA lesions.

At the end of levodopa treatment (day 22, before receiving sarizotan or vehicle), no significant differences were observed in the total dyskinesia score between the four groups of animals (three groups receiving sarizotan and the control group; data not shown). On day 23, the subthalamic administration of sarizotan at the higher doses tested (10 ng and 1 μg) significantly decreased the total dyskinesia score in levodopa-treated rats in comparison with the rats treated only with levodopa ($p<0.05$, $p<0.01$, respectively; Fig. 3a).

The time course curve evidences similar pattern of total dyskinetic movements in all groups on day 22 of levodopa treatment before sarizotan or vehicle administration (Fig. 3b). On day 23, the time course curve shows the decrease ($p<0.01$; Fig. 3b) in the total levels of dyskinesia after sarizotan administration which started early (10 min) after drug administration.

Axial dyskinesia

At the end of levodopa treatment (day 22, before receiving sarizotan or vehicle), no significant differences were

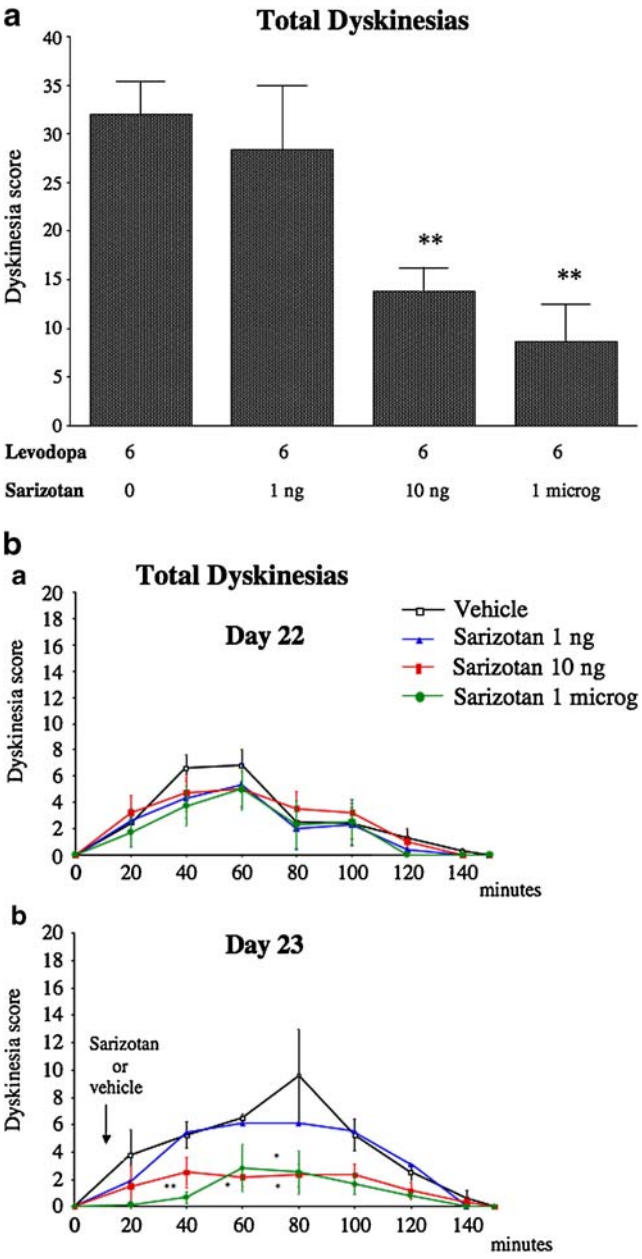

Fig. 3 **a** Effect of acute subthalamic administration of sarizotan on the total dyskinesias induced by chronic levodopa treatment (6 mg/kg with 15 mg/kg benserazide, i.p., twice a day) on day 23. Sarizotan significantly attenuated levodopa-induced dyskinesias at the highest doses tested (10 ng and 1 μg). **b** Time course of the overall dyskinesias on day 22 after levodopa treatment (*A*) and day 23 after sarizotan administration (*B*). *$p<0.05$, **$p<0.01$ vs levodopa alone. Data are represented as mean±SEM

observed in the axial dyskinesia score between the four groups of animals (three groups receiving sarizotan and the control group; data not shown).

On day 23, the subthalamic administration of sarizotan at the higher doses tested (10 ng and 1 μg) decreased the axial dyskinesia score in levodopa-treated rats in comparison with rats treated only with levodopa ($p<0.05$, $p<0.01$, respectively; Fig. 4a). The time course curve shows the decrease in the axial dyskinesia after sarizotan administration (Fig. 4b).

Orolingual dyskinesia

At the end of levodopa treatment (day 22, before receiving sarizotan or vehicle), no significant differences were observed in the orolingual dyskinesia score between the four groups of animals (three groups receiving sarizotan and the control group; data not shown).

On day 23, the subthalamic administration of sarizotan at the higher doses tested (10 ng and 1 μg) shows a tendency to decrease the orolingual dyskinesia score in levodopa-treated rats, achieving statistical significance in the group

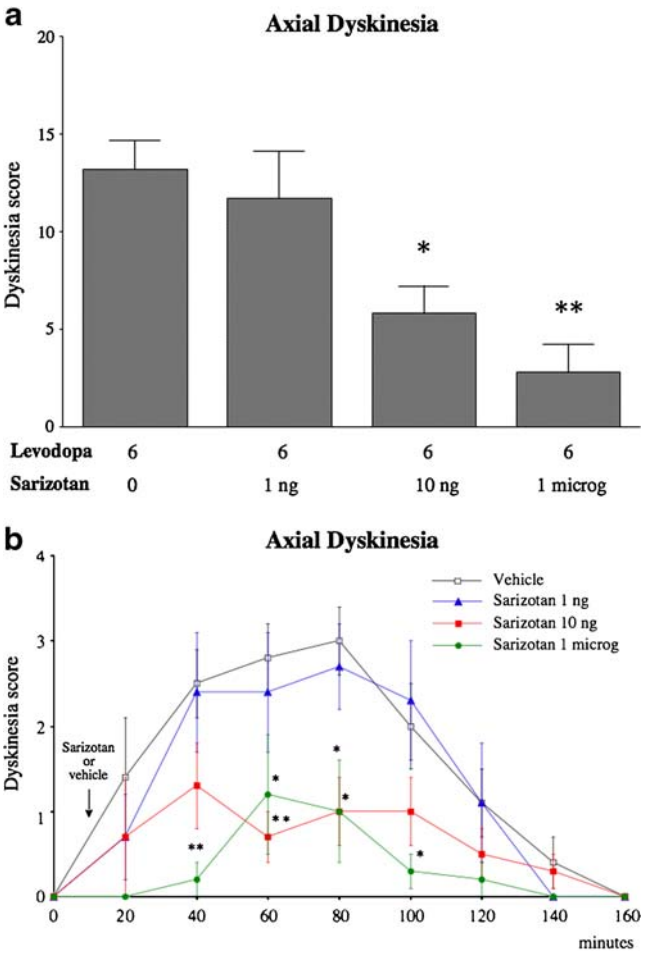

Fig. 4 **a** Effect of acute subthalamic administration of sarizotan on axial dyskinesias induced by chronic levodopa treatment (6 mg/kg with 15 mg/kg benserazide, i.p., twice a day). Sarizotan significantly attenuated levodopa-induced dyskinesias at the highest doses tested (10 ng and 1 μg). **b** Time course of the axial dyskinesias on day 23 after sarizotan administration. *$p<0.05$, **$p<0.01$ vs levodopa alone. Data are represented as mean±SEM

that received 10 ng of sarizotan (Fig. 5a). The time course curve shows the decrease in the orolingual dyskinesia after the subthalamic administration of the higher dose of sarizotan (Fig. 5b).

Limb dyskinesia

At the end of levodopa treatment (day 22, before receiving sarizotan or vehicle), no significant differences were observed in the limb dyskinesia score between the four groups of animals (three groups receiving sarizotan and the control group; data not shown).

On day 23, the subthalamic administration of sarizotan at the higher doses tested (10 ng and 1 μg) decreased the limb dyskinesia score in levodopa-treated rats in comparison with rats treated only with levodopa ($p<0.05$, $p<0.01$, respectively; Fig. 6a). The time course curve shows the decrease in the limb dyskinesia after sarizotan administration (Fig. 6b).

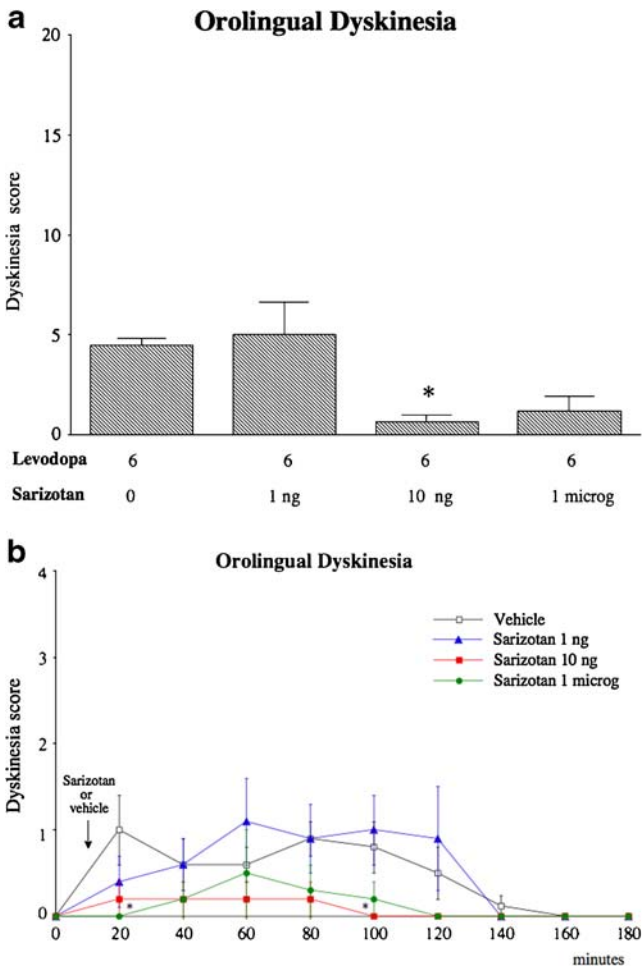

Fig. 5 **a** Effect of acute subthalamic administration of sarizotan on orolingual dyskinesias induced by chronic levodopa treatment (6 mg/kg with 15 mg/kg benserazide, i.p., twice a day). Sarizotan significantly attenuated levodopa-induced dyskinesias when administered at the dose of 10 ng. **b** Time course of the orolingual dyskinesias on day 23 after sarizotan administration. *$p<0.05$ vs levodopa alone. Data are represented as mean±SEM

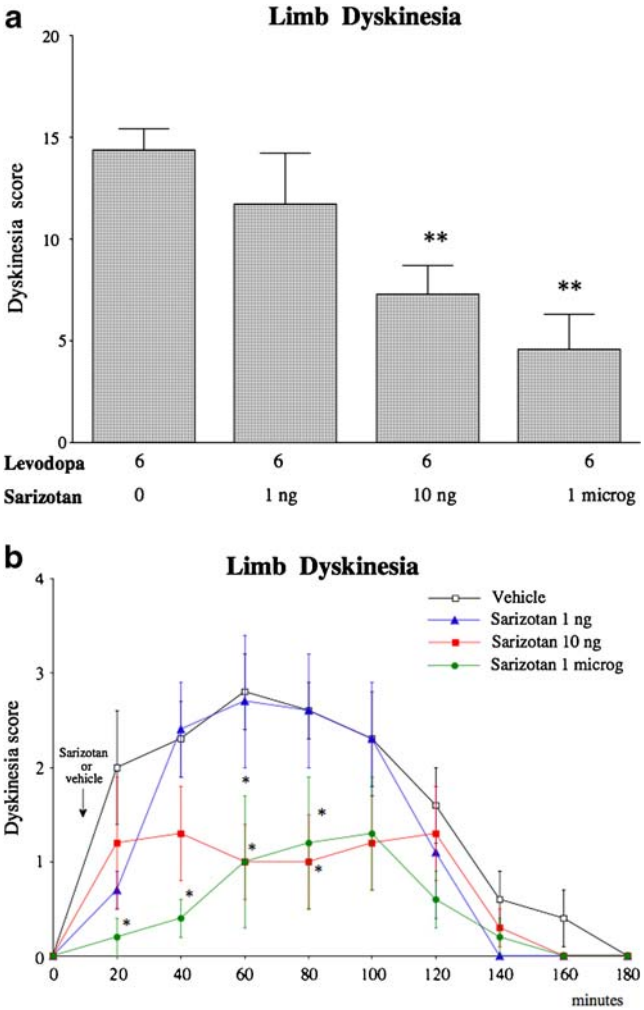

Fig. 6 **a** Effect of acute subthalamic administration of sarizotan on limb dyskinesias induced by chronic levodopa treatment (6 mg/kg with 15 mg/kg benserazide, i.p., twice a day). Sarizotan significantly attenuated levodopa-induced dyskinesias at the highest doses tested (10 ng and 1 μg). **b** Time course of the limb dyskinesias on day 23 after sarizotan administration. *$p<0.05$, **$p<0.01$ vs levodopa alone. Data are represented as mean±SEM

Discussion

We found that local administration of sarizotan into the STN of 6-OHDA-lesioned rats resulted in marked attenuation of levodopa-induced dyskinesias. This shows for the first time that levodopa-induced dyskinesias can be pharmacologically modulated at the level of the STN probably by stimulation of the 5-HT_{1A} receptors. All AIM subtypes

evaluated, such as axial, limb, and orofacial, demonstrated similar reductions. The antidyskinetic effect of the subthalamic administration of sarizotan was not associated with any decrease of motoric response such as rotational behavior (data not shown).

It is now well known that an increase in serotoninergic transmission can contribute to the appearance of dyskinesias (Carta et al. 2007; Kish et al. 2008). Moreover, it has been recently described that serotonin mesencephalic neuron transplants exacerbate levodopa-induced dyskinesia in 6-OHDA-lesioned rats (Carlsson et al. 2007). It is thus conceivable that inhibition of serotonin release as a consequence of 5-HT_{1A} agonist action of sarizotan might alleviate dyskinesias (Ba et al. 2007; Bibbiani et al. 2001; Bishop et al. 2006; Carta et al. 2007; Dekundy et al. 2007; Dupre et al. 2008; Eskow et al. 2007; Lunblad et al. 2005). The present results extend previous findings in MPTP monkeys (Bedard et al. 2006; Bibbiani et al. 2001) and PD patients (Bara-Jimenez et al. 2005; Olanow et al. 2004) where systemic administration of the 5-HT_{1A} agonist sarizotan can attenuate levodopa-induced dyskinesias without worsening parkinsonian symptoms.

The exact mechanism that underlies the antidyskinetic effect of sarizotan is still unknown. Recently, it has been demonstrated that sarizotan has no effect on the pharmacokinetics of levodopa (Krösser et al. 2007). It has been suggested that the antidyskinetic properties of sarizotan could be mediated via its 5-HT_{1A} agonist action in the motor cortex, reducing the activity of the corticostriatal glutamate pathway (Antonelli et al. 2005). However, while the mechanism for the antidyskinetic action of sarizotan is still unclear, the most prominent theory postulates that in the DA-depleted brain, serotoninergic raphestriatal neurons usurp the role of the nigrostriatal pathway, converting levodopa into DA and releasing it into the striatum (Arai et al. 1996; Tanaka et al. 1999). Stimulating 5-HT_{1A} somatodendritic autoreceptors on raphestriatal neurons decreases pulsatile stimulation of DA receptor in the striatum and prolongs DA half-life (Tanaka et al. 1999; Kannari et al. 2001). However, recent evidence also indicates that 5-HT_{1A} stimulation may also directly influence postsynaptic DA-receptor-mediated behaviors that are raphe-independent, since it has been recently demonstrated that 5-HT_{1A} stimulation reduced AIMs induced by selective D1 or D2 DA agonists in the 6-OHDA model in rats (Dupre et al. 2007). The involvement of the DA D3 properties of sarizotan in the suppression of dyskinesias has also been demonstrated in rats (Bartoszyk et al. 2006; Gerlach et al. 2006a, b).

Until now, the striatum has been the structure of the basal ganglia considered a possible target for the antidyskinetic effect of sarizotan (Bibbiani et al. 2001; Dupre et al. 2007). However, Bartoszyk (2006) proposed an integrated basal ganglia circuitry model as working hypothesis for the mechanism of antidyskinetic action of sarizotan involving at least 5-HT1A and D3 receptors. Our results show the relevant role of the stimulation of the 5-HT_{1A} receptors in the STN for the antidyskinetic action of sarizotan. The STN is a glutamatergic nucleus of the basal ganglia that is considered to be a major driving force in the basal ganglia circuit (Albin et al. 1989; DeLong 1990) and is involved in motor functions (DeLong 1990; DeLong et al. 1985; Matsumara et al. 1992) as well as movement disorders (Albin et al. 1989; Bergman et al. 1994; Hamani et al. 2004; Wichmann and DeLong 1996, 2003). Levodopa-induced dyskinesias are physiologically characterized by a decreased firing frequency, abnormal firing patterns, and changes in the oscillatory activity of the STN (Alonso-Frech et al. 2006; Levy et al. 2001; Vitek and Giroux 2000).

The STN is innervated by rich 5-HT fibers (Bobillier et al. 1976; Mori et al. 1985; Lavoie and Parent 1990) mainly from the neurons in the dorsal raphe nucleus (Bobillier et al. 1976; Lavoie and Parent 1990). Several subtypes of 5-HT receptors are expressed in the STN. Histochemical studies by in situ hybridization have shown that 5-HT_{2C} and 5-HT_4 receptor mRNA is present at very high levels in the STN, whereas 5-HT_{1A} and 5-HT_{2A} receptor mRNA appears to be expressed at lower levels (Pompeiano et al. 1994; Wright et al. 1995).

Extracellular single-unit recordings in mouse brain slices were used to determine the effect of exogenously applied 5-HT on STN neurons. In STN neurons, 5-HT elicits two distinct effects, at times on the same neuron, the first being an excitation which is mediated by 5-HT_{2C} and 5-HT_4 receptors and the second an inhibition which is mediated by 5-HT_{1A} receptors (Standford et al. 2005). It is interesting to note that 5-HT_{2C}, 5-HT_4, and 5-HT_{1A} receptors appear to regulate both resting and voltage-gated potassium conductances (Andrade and Nicoll 1987; Davies et al. 1987; Xiang and Kitai 2005). 5-HT_{1A} receptor activation which increases cAMP presumably causes an opening of potassium conductance as reported in other cell types (Andrade and Nicoll 1987; Davies et al. 1987). Thus, it is conceivable to hypothesize that the 5-HT_{1A} stimulation induced by STN administration of sarizotan may influence neuronal excitability in the STN and thus modify its firing pattern, reducing levodopa-induced dyskinesia. This hypothesis is supported by the recent observation that a lesion of the dorsal raphe nucleus alters neuronal activity of the STN in 6-OHDA rats (Liu et al. 2007). On the other hand, STN activity is also regulated by glutamatergic inputs from the cerebral cortex, thalamus, pedunculopontine nucleus (Breit et al. 2006; Lanciego et al. 2004; Nambu et al. 2000), and also by dopaminergic projections from the substantia nigra (Cragg et al. 2004; Hassani et al. 1997). We cannot exclude that sarizotan-mediated activation of 5-HT_{1A} receptors could also modulate some of these afferent projections to

the STN and participate in the antidyskinetic effect here reported. In addition, since it has been shown that D3 and D4 DA receptors also play a role in the effects induced by sarizotan (Bartoszyk et al. 2004; Kuzhikandathil and Bartoszyk 2006) and that the activation of these receptors may modulate the STN activity (Hernández et al. 2006), it is necessary to take into account a possible effect of these receptors in the antidyskinetic properties of sarizotan. Further research is required in order to investigate the mechanisms involved in the antidyskinetic effect of sarizotan.

In any case, our findings open up a novel possibility to understand the pathophysiology and develop newer treatments for levodopa-induced dyskinesias. For instance, levodopa-induced dyskinesias are diminished in PD patients treated with deep brain stimulation of the STN (DBS-STN), which has most commonly been attributed to a levodopa sparing effect of this technique. However, such beneficial effect can be seen in patients in whom levodopa is not modified or even increased. Our data now may be taken to suggest a putative pre-synaptic effect of DBS on serotonin release. Clearly, further research into the direct action of sarizotan and other drugs (i.e., serotoninergic, dopaminergic) onto STN activity and dyskinesias is warranted.

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