

## Study of the Antidyskinetic Effect of Eltoprazine in Animal Models of Levodopa-Induced Dyskinesia

Erwan Bezard, PhD,<sup>1,2,3</sup> Elisabetta Tronci, PhD,<sup>4,5</sup> Elsa Y. Pioli, PhD,<sup>6</sup> Qin Li, PhD,<sup>3,6</sup> Gregory Porras, PhD,<sup>6</sup> Anders Björklund, MD,<sup>5</sup> and Manolo Carta, PhD<sup>4,5\*</sup>

<sup>1</sup>Institute for Neurodegenerative Diseases, Bordeaux University, Bordeaux, France

<sup>2</sup>Institute for Neurodegenerative Diseases, National Center for Scientific Research, Bordeaux, France

<sup>3</sup>Institute of Laboratory Animal Sciences, China Academy of Medical Sciences, Beijing, China

<sup>4</sup>Physiology Section, Department of Biomedical Sciences, Cagliari University, Cagliari, Italy

<sup>5</sup>Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden

<sup>6</sup>Motac Neuroscience Ltd., Manchester, United Kingdom

The serotonin (5-hydroxytryptamine [5HT]) system has recently emerged as an important player in the appearance of L-3,4-dihydroxyphenylalanine (levodopa [L-dopa])–induced dyskinesia in animal models of Parkinson's disease. In fact, dopamine released as a false transmitter from serotonin neurons appears to contribute to the pulsatile stimulation of dopamine receptors, leading to the appearance of the abnormal involuntary movements. Thus, drugs able to dampen the activity of serotonin neurons hold promise for the treatment of dyskinesia. The authors investigated the ability of the mixed 5-HT 1A/1B receptor agonist eltoprazine to counteract L-dopa–induced dyskinesia in 6-hydroxydopamine-lesioned rats and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaques. The data demonstrated that eltoprazine is extremely effective in suppressing dyskinesia in experimental models, although this effect was accompanied by a partial worsening of the therapeutic effect of L-dopa.

Interestingly, eltoprazine was found to (synergistically) potentiate the antidyskinetic effect of amantadine. The current data indicated that eltoprazine is highly effective in counteracting dyskinesia in preclinical models. However, the partial worsening of the L-dopa effect observed after eltoprazine administration represents a concern; whether this side effect is due to a limitation of the animal models or to an intrinsic property of eltoprazine needs to be addressed in ongoing clinical trials. The data also suggest that the combination of low doses of eltoprazine with amantadine may represent a valid strategy to increase the antidyskinetic effect and reduce the eltoprazine-induced worsening of L-dopa therapeutic effects. © 2013 Movement Disorder Society

**Key Words:** dyskinesia; levodopa; eltoprazine; serotonin; amantadine

The appearance of dyskinesia upon chronic treatment with L-3,4-dihydroxyphenylalanine (levodopa [L-dopa]) is a major problem for the management of the motor symptoms in patients with Parkinson's dis-

ease (PD). The N-methyl-D-aspartic acid (NMDA) receptor antagonist amantadine is the only drug used in patients to control dyskinesia, with limited efficacy and side effects.<sup>1</sup> Therefore, there is a need for the

Additional Supporting Information may be found in the online version of this article.

\*Correspondence to: Dr. Manolo Carta, Department of Biomedical Sciences, Section of Physiology, Cagliari University, University Campus, 09042 Monserrato, Italy; manolocarta@unica.it

**Funding agencies:** This study was supported by a grant from the Michael J. Fox Foundation. E.B. was also supported by grants from the Agence Nationale de la Recherche, the China Science Fund, the Seventh Framework Programme (FP7 of the European Union, France Parkinson, Fondation de France, and the Cariplo Foundation.

**Relevant conflicts of interest/financial disclosures:** E.B. has an equity stake in Motac Holding Ltd. and receives consultancy payments from Motac Neuroscience Ltd. E.B., Q.L., and G.P. are employees of Motac Neuroscience Ltd.

Full financial disclosures and author roles may be found in the online version of this article.

**Received:** 24 August 2012; **Revised:** 19 December 2012; **Accepted:** 21 December 2012

**Published online 00 Month 2013 in Wiley Online Library (wileyonlinelibrary.com).**

**DOI:** 10.1002/mds.25366

development of novel pharmacological therapies to control dyskinesia and prolong the beneficial effects of L-dopa.<sup>2</sup>

L-dopa is very efficient during the first years of administration, conceivably due to the ability of spared dopamine (DA) neurons to convert L-dopa into DA, mediate regulated synaptic release, and maintain physiological DA receptor stimulation at striatal neurons. Recently, the serotonin (5-hydroxytryptamine [5HT]) system has emerged as an important player in the appearance of L-dopa-induced dyskinesia (LID).<sup>3–5</sup> Serotonin neurons can convert exogenous L-dopa to DA, store DA in synaptic vesicles, and release it in an activity-dependent manner. However, serotonin neurons lack the autoregulatory feedback mechanism able to fine-tune DA levels in the synaptic cleft, as provided in DA neurons by D2 receptors and DA transporter. Therefore, in a situation of advanced DA neurodegeneration, L-dopa-derived DA released as a false neurotransmitter from serotonin terminals leads to excessive stimulation of hypersensitive striatal DA receptors. Accordingly, toxin lesion or silencing of serotonin neurons by drugs acting on 5-HT1 autoreceptors have been shown to suppress LID, both in parkinsonian rats<sup>3</sup> and monkeys.<sup>4</sup> Interestingly, simultaneous activation of 5-HT1A and 5-HT1B receptors induced a potent synergistic effect on the suppression of dyskinesia.<sup>3,4</sup>

Drugs acting on the 5-HT1A receptor alone have been explored in dyskinetic patients; however, at doses that do not interfere with the therapeutic efficacy of L-dopa, the effect on dyskinesia has been mild or non-significant.<sup>6,7</sup> Our recent reports suggest that drugs acting simultaneously on the 2 autoreceptors could be more efficacious. Eltoprazine, which is a mixed 5-HT1A/B agonist that was developed by Solvay Pharmaceuticals, Inc. (Marietta, GA) for the treatment of aggression, is a promising candidate. This drug exhibits a safe toxicological profile and lack of serious side effects,<sup>8,9</sup> and it is currently in use in a clinical trial in patients with attention deficit hyperactivity disorder (ClinicalTrials.gov Identifier: NCT01266174). It is worth mentioning that, in contrast to other 5-HT1A receptor agonists such as sarizotan and buspirone, eltoprazine is not known to have any antagonistic affinity for the DA receptors.<sup>10–12</sup> In this report, we demonstrate that eltoprazine is highly effective in blocking LID both in 6-hydroxydopamine (6-OHDA)-lesioned rats and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaques, although this effect is accompanied by a significant reduction in the therapeutic efficacy of L-dopa. Moreover, we show that eltoprazine and amantadine act synergistically against LID, suggesting the possibility that the use of these 2 drugs in combination may increase their clinical efficacy against dyskinesia.

## Materials and Methods

### Rat Study

Adult female Sprague–Dawley rats (225–250 g; B&K Universal AB, Sollentuna, Sweden) were housed under a 12-hour light/12-hour dark cycle with free access to water and food. All surgical procedures were performed according to the regulations set by the Ethical Committee for Use of Laboratory Animals at Lund University.

6-OHDA was administered unilaterally into the medial forebrain bundle, as previously reported (single injection of 14 µg free base in 4 µL)<sup>3</sup>. Three weeks later, the rats were screened in the amphetamine-induced rotation test (2.5 mg/kg). Animals that exhibited  $\geq 6$  full body turns per minute ipsilateral to the lesion side were included in the study. The animals used for acute experiments were treated with L-dopa (6 mg/kg subcutaneously plus 10 mg/kg benserazide) daily for 3 weeks, until a stable level of abnormal involuntary movements (AIMs) was achieved. The animals were then allocated into 3 groups, which were balanced according to their AIMs scores, and then challenged with L-dopa either alone or in combination with eltoprazine at 2 doses. An additional group of 6-OHDA-lesioned animals was primed with daily injections of apomorphine (0.025 mg/kg subcutaneously) for 2 weeks and then challenged with apomorphine either alone or in combination with eltoprazine at 2 doses.

For the chronic study, 6-OHDA-lesioned, L-dopa-naïve rats were allocated into 3 groups balanced on the basis of their amphetamine-induced rotation scores. These groups received daily injections of either L-dopa alone or in combination with eltoprazine for 4 weeks. Eltoprazine was also given chronically to already dyskinetic rats for 6 weeks, whereas control animals received L-dopa only.

### Behavioral Analysis

#### *Apomorphine and L-Dopa-Induced AIMs*

AIMs were evaluated based on the rat dyskinesia scale according to previous procedures and methods.<sup>3,4,13</sup>

#### *Activity Test*

Locomotor activity was assessed in a separate group of animals in open-field chambers.<sup>14</sup>

#### *Stepping Test*

The stepping test was performed as previously described.<sup>15,16</sup> Baseline values for the impaired paw were obtained, and only rats scoring 0 or 1 steps were included in the study. On the first day of the drug treatment, rats were tested twice at 50 minutes after saline or drug administration. Values are reported as average of the 2 sessions.

### Immunohistochemistry

The animals employed in the chronic study were killed 48 hours after the last injection, and their brains were removed and processed for tyrosine hydroxylase immunohistochemistry (to verify the dopaminergic lesion).<sup>14</sup>

### Monkey Study

Six female cynomolgus monkeys (*Macaca fascicularis*; Xierxin, Beijing, People's Republic of China) were used. All animals were 5 years old, had a mean  $\pm$  standard deviation body weight of  $3.3 \pm 0.3$  kg, and were previously treated with other drugs. However, a 2-month washout with daily exposure to L-dopa was undertaken before this study to avoid possible interactions. The monkeys were housed in individual primate cages under controlled conditions of humidity, temperature, and light (12-hour light/12-hour dark cycle; lights on at 8:00 AM); food and water were available ad libitum in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility. Animal care was supervised by veterinarians skilled in the health care and maintenance of nonhuman primates. Experiments were carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals.

### Experimental Parkinsonism and Dyskinesia

Experiments were conducted according to previously published procedures and methods.<sup>14,17-21</sup> L-dopa/carbidopa (or its vehicle) and amantadine (or its vehicle) were administered orally, whereas eltoprazine (or its vehicle) was administered subcutaneously 15 minutes before L-dopa/vehicle administration in the animal's home cage. The animals were immediately transferred to an observation cage after oral administration (dimensions: 1.1 m  $\times$  1.5 m  $\times$  1.1 m) for a 250-minute behavioral assessment (see below). Two sets of experiments were performed. The first series aimed at defining the active doses of eltoprazine against LID. Eltoprazine doses ranged from 0.3 to 1.0 mg/kg. The second series investigated the effects of the combination of suboptimal doses of eltoprazine and amantadine. The following treatments were employed: L-dopa/carbidopa-vehicle, L-dopa/carbidopa, and eltoprazine (0.5, 0.6, 0.7, and 0.8 mg/kg); L-dopa/carbidopa plus amantadine (10 and 20 mg/kg); and L-dopa/carbidopa plus eltoprazine (0.5, 0.6, 0.7 and 0.8 mg/kg) plus amantadine (10 mg/kg). The different treatments were tested using a Latin square design.

### Behavioral Assessment

A battery of behavioral tests was performed as previously described.<sup>14,17,19-22</sup> A quantitative assessment of locomotor activity using computer-based, passive,

infrared activity monitors (Excalibur; modified by the Central Electronic Workshop, University of Manchester, Manchester, United Kingdom) was obtained every 5 minutes for 250 minutes. Nonparametric measures based on range of movement, bradykinesia, and posture scales were made; and parkinsonian condition (and its reversal) was assessed on a parkinsonian monkey rating scale by post hoc analysis of video recordings by observers blinded to the treatment in 10-minute observation periods every 30 minutes for 250 minutes, as previously described.<sup>14,17,19-21</sup>

The severity of dyskinesia was rated using the Dyskinesia Disability Scale<sup>14,17,19-21</sup> by post hoc analysis of video recordings in 10-minute observation periods every 30 minutes for 250 minutes. Both choreic (hyperkinetic, purposeless, dance-like movements) and dystonic (sustained, abnormal muscle contractions) components of dyskinesia were rated as reported previously.<sup>14</sup>

The duration of anti-parkinsonian action, ON-time, was defined as the number of minutes for which the bradykinesia score is zero. In addition, the duration of ON-time associated with dyskinesia of varying severity is calculated as follows: *Good ON-time* represents the number of minutes for which the bradykinesia score is zero while dyskinesia is absent, mild, or moderate, i.e., it commands a score of 2 or less. *Bad ON-time* represents the number of minutes for which the bradykinesia score is zero while the dyskinesia score is marked or severe, i.e., greater than 2.<sup>23,24</sup>

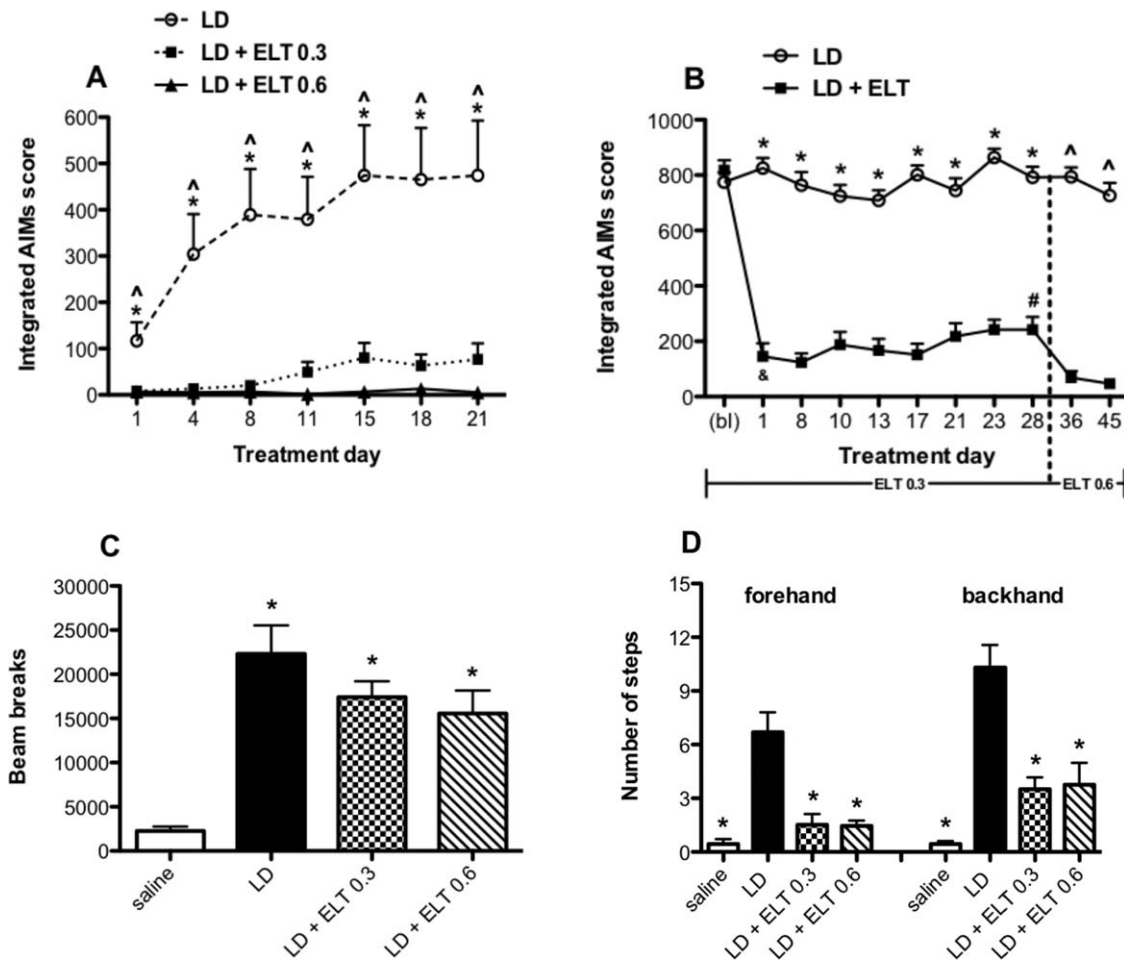
### Statistical Analysis

Statistical analysis of rat data was performed using Statistica software (StatSoft, Inc, Tulsa, OK). Significance between groups was evaluated using 1-way or 2-way analysis of variance (ANOVA) followed by a Newman-Keuls or Tukey's multiple comparison test for the rat study. Behavioral data in the macaque model were analyzed using Friedman followed by Dunn's multiple comparisons test for nonparametric ratings and using a 1-way, repeated measures ANOVA followed by a Dunnett test for parametric activity counts-derived data. Statistical significance was set at  $P < 0.05$ .

## Results

### Acute Effect of Eltoprazine on LID in Parkinsonian Rats

Twenty-seven 6-OHDA-lesioned, L-dopa-primed, dyskinetic rats were allocated into 3 groups, which were balanced according to their dyskinesia score, and were subjected to a drug challenge with either L-dopa alone (6 mg/kg plus benserazide 10 mg/kg subcutaneously) or in combination with subcutaneous eltoprazine at 0.3 and 0.6 mg/kg doses. Significant reductions of 83% and 99% compared with the control group were observed at 0.3 and 0.6 mg/kg eltoprazine doses,



**FIG. 1.** (A) Drug-naïve 6-hydroxydopamine (6-OHDA)-lesioned rats were subjected to daily treatment with L-dopa (LD) (6 mg/kg plus benserazide 10 mg/kg, subcutaneously), either alone or in combination with eltoprazine (ELT) 0.3 or 0.6 mg/kg, for 3 weeks. A 2-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test revealed that both LD plus ELT 0.3 mg/kg and LD plus ELT 0.6 mg/kg treatments resulted in significant protection from the development of dyskinesia compared with LD alone ( $P < 0.05$ ). (B) Two groups of LD-primed, highly dyskinetic rats were subjected to a daily treatment with LD alone or LD plus ELT 0.3 mg/kg. A 2-way ANOVA followed by Tukey's multiple comparison test revealed that treatment with LD plus ELT 0.3 mg/kg resulted in a significant and permanent reduction of dyskinesia on a 4-week treatment compared with the LD-only group ( $P < 0.05$ ). Moreover, treatment with LD plus ELT 0.6 mg/kg for an additional 2 weeks induced a stronger reduction of dyskinesia ( $P < 0.05$ ). (C) The effect of ELT on LD-induced motor activation is illustrated. A 1-way ANOVA followed by a Newmann-Keuls multiple comparison test revealed that neither dose of ELT reduced the ability of LD to improve horizontal activity ( $P < 0.05$ ). (D) The effect of ELT on LD-induced forelimb use in the stepping test is illustrated. A 1-way ANOVA followed by a Newmann-Keuls multiple comparison test revealed that both doses of ELT significantly reduced the ability of LD to increase the number of adjusting steps of the parkinsonian forelimb ( $P < 0.05$ ).

respectively (mean integrated AIMs score: 655 for controls, 111 for eltoprazine 0.3 mg/kg, 6 for eltoprazine 0.6 mg/kg; 1-way ANOVA,  $P < 0.05$ ).

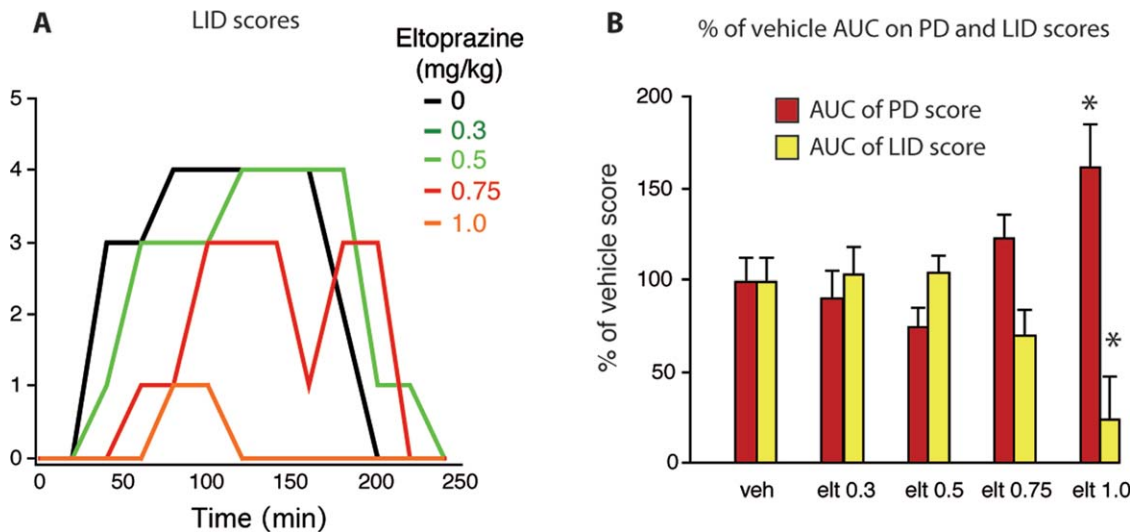
### Acute Effect of Eltoprazine on Apomorphine-Induced Dyskinesia in Parkinsonian Rats

To establish whether activation of postsynaptic 5-HT<sub>1</sub> receptors may account at least in part for the effect of eltoprazine on LID, 18 6-OHDA-lesioned, apomorphine-primed, dyskinetic rats were allocated into 3 well balanced groups according to their dyskinesia score and were subjected to a drug challenge either with apomorphine alone (0.025 mg/kg subcutaneously) or in combination with eltoprazine at 0.3 and 0.6 mg/kg doses. The results showed that eltoprazine produced only a weak, nonsignificant reduction of apomorphine-

induced dyskinesia (mean integrated AIMs score: 364 for controls, 323 for eltoprazine 0.3 mg/kg, 296 for eltoprazine 0.6 mg/kg; 1-way ANOVA,  $P > 0.05$ ), pointing to a presynaptic effect of eltoprazine against LID.

### Effect of Chronic Treatment with Eltoprazine on LID in Parkinsonian Rats

Long-term treatment with 5-HT<sub>1</sub> agonists raises concerns for possible desensitization of the presynaptic receptors, which may result in an overtime reduction of their efficacy. To investigate this, drug-naïve, 6-OHDA-lesioned rats were allocated into 3 groups ( $n = 11$  per group), balanced according to their amphetamine-induced rotation score, and were subjected to daily treatment with L-dopa (6 mg/kg plus benserazide 10 mg/kg, subcutaneously) either alone or in



**FIG. 2.** (A) This time-course panel reports the dyskinesia rating (median) after L-dopa and eltoprazine (elt) (0–1.0 mg/kg). Administration of L-dopa and eltoprazine started at  $t=0$  minutes. (B) The area under the curve (AUC) of dyskinesia (LID) and parkinsonian disability (PD) scores after L-dopa and eltoprazine (0–1.0 mg/kg) are expressed as a percentage of the L-dopa plus vehicle (veh) condition (vs. vehicle treatment [\*]).

combination with eltoprazine at 0.3 and 0.6 mg/kg doses, for 3 weeks (phase 1). Eltoprazine resulted in significant protection from the development of dyskinesia at both doses (84% and 99% reduction at 0.3 and 0.6 mg/kg doses, respectively, compared with the control group) (Fig. 1A).

Clinical investigation of the effect of 5-HT<sub>1</sub> agonists likely will be performed first in already dyskinetic patients. Therefore, it was relevant to know whether the effect of eltoprazine was maintained upon chronic treatment in already dyskinetic rats. Two groups of L-dopa-primed, highly dyskinetic animals ( $n=12$  per group) were subjected to daily treatment with either L-dopa alone or L-dopa plus eltoprazine at the 0.3 mg/kg dose (phase 2). Eltoprazine treatment resulted in a significant and permanent reduction of dyskinesia upon 4-week treatment compared with the control group (70% reduction of AIMs compared with the control group at the last L-dopa treatment) (Fig. 1B). Importantly, no loss of efficacy was seen during this time. Eltoprazine was then escalated to 0.6 mg/kg for an additional 2 weeks, and near-to-full suppression of LID was observed (93% reduction compared with the control group at the last L-dopa treatment) (Fig. 1B).

### Effect of Eltoprazine on L-Dopa-Induced Motor Activity and Forelimb Use in the Stepping Test

A group of L-dopa-naive, 6-OHDA-lesioned rats was employed to test the impact of eltoprazine on the therapeutic efficacy of L-dopa in the motor activity and stepping tests. The results showed that eltoprazine did not reduce the L-dopa-induced motor activity (Fig. 1C). Moreover, eltoprazine did not reduce the basal activity of the animals OFF L-dopa (not shown).

However, an almost complete suppression of the therapeutic effect was seen in the stepping test after eltoprazine administration (Fig. 1D).

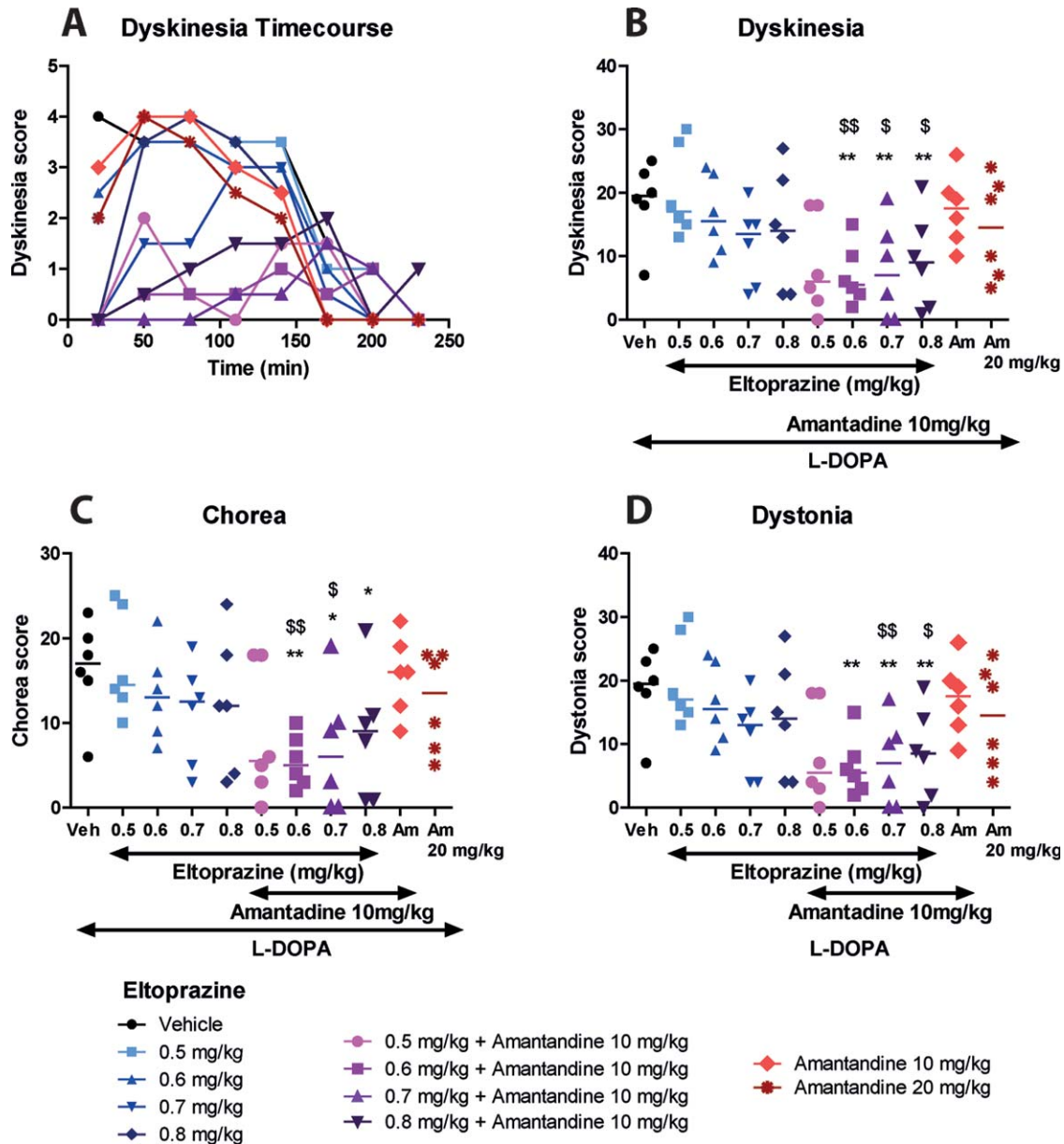
### Effect of Eltoprazine on LID in MPTP-Treated Macaques

Five dyskinetic, L-dopa-primed, MPTP-treated macaques were used to test the effect of eltoprazine on LID. Each animal was subjected to administration of L-dopa (at the minimal dose that produced the maximal anti-parkinsonian effect), either alone or in combination with eltoprazine at different doses. Dyskinesia and parkinsonism were evaluated according to modified rating scales, as previously described.<sup>18,19</sup>

Figure 2A shows that eltoprazine resulted in a dose-dependent reduction of LID, with near-complete suppression at the highest dose (about 90% reduction in the area under the curve [AUC] at a 1.0 mg/kg dose) (Fig. 2B). However, this effect was accompanied by a significant reduction in the anti-parkinsonian efficacy of L-dopa (Fig. 2B). Although significant, such an important reduction has to be assessed in the light of actual scores. In the OFF state, animals displayed a score of  $9 \pm 0.5$  (median  $\pm$  standard error of the mean). This was reversed by L-dopa, with animals reaching scores of  $0 \pm 0.2$  at 100 minutes post-administration, whereas L-dopa plus eltoprazine 1.0 mg/kg produced scores of  $2.0 \pm 0.6$ . Therefore, animals still were improved by L-dopa treatment but at lower magnitude when given in combination with eltoprazine.

### Effect of Eltoprazine plus Amantadine on LID in MPTP-Treated Macaques

The worsening of the therapeutic effect of L-dopa observed after administration of eltoprazine is a



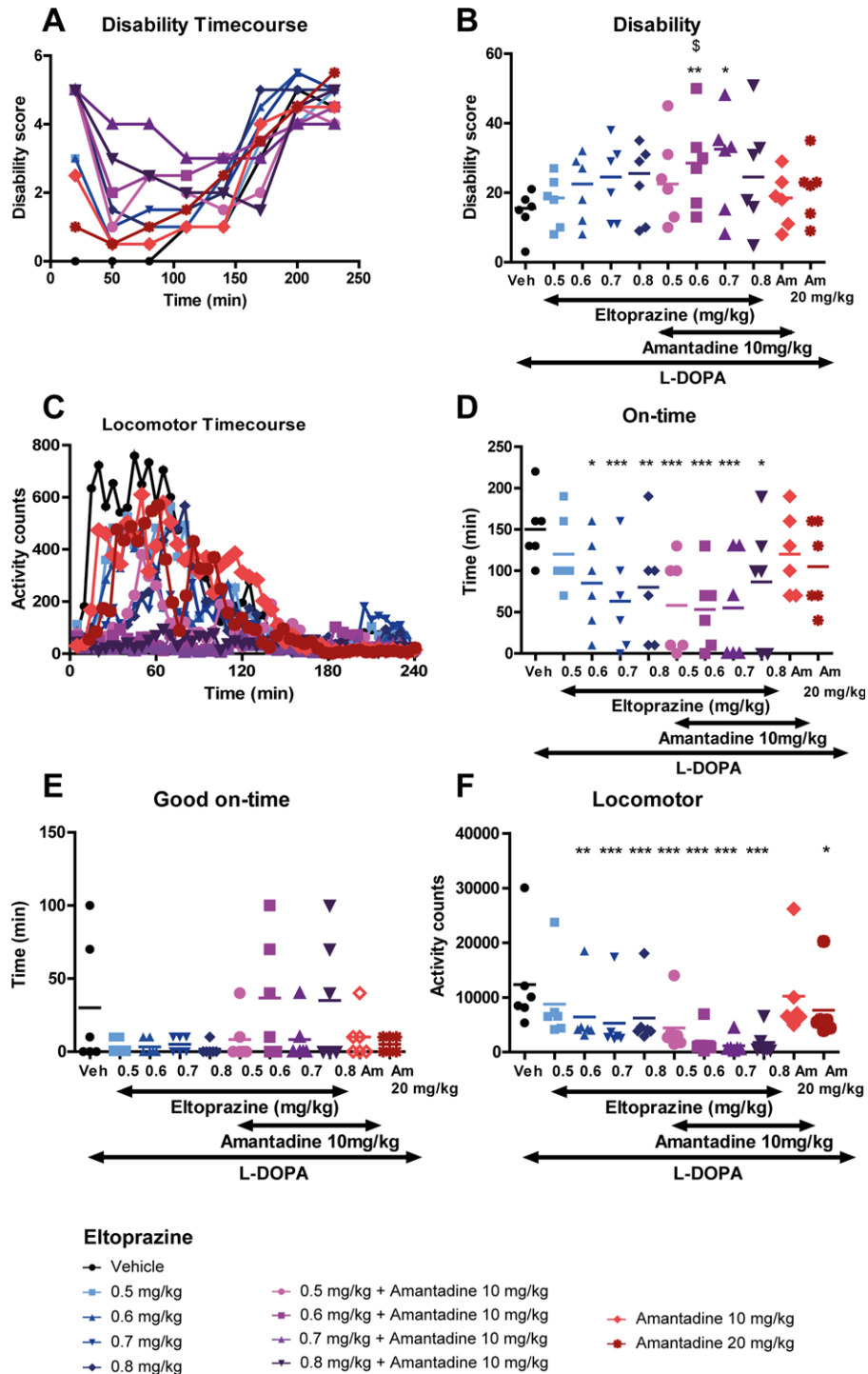
**FIG. 3.** The impact of drug treatments on dyskinesia severity is illustrated as (A) time-course scores (median scores are shown without standard error of the mean for readability) and (B) area under the curve (AUC) dyskinesia scores, as well as the (C) chorea and (D) dystonia subscores. Administration of L-dopa, eltoprazine, or amantadine started at  $t=0$  minutes (\*, vs. vehicle treatment; \$, vs. amantandine 10 mg/kg).

concern for clinical application. Moreover, any new antidyskinetic therapy should be superior to amantadine to justify its clinical use. Thus, a second experiment was performed in MPTP-treated macaques to directly compare the effect of eltoprazine and amantadine. In addition, these drugs also were combined at subthreshold doses to investigate possible addictive/synergistic effects. Six MPTP-treated macaques were used (Fig. 3). Only a trend toward reduction was observed in the dyskinesia score after individual administration of eltoprazine (Fig. 3A,B), in line with the first eltoprazine test, in which marked antidyskinetic efficacy was seen only after administration of eltoprazine at 1.0 mg/kg. However, a clear synergistic

effect against LID was seen after combination of the 2 compounds at low doses, particularly for eltoprazine 0.6 mg/kg plus amantadine 10 mg/kg.

When dissecting the different components of dyskinesia, the synergistic effect appeared particularly striking on both *chorea* and *dystonia* (Fig. 3C,D). A partial worsening of *disability* was seen after the combined drugs (Fig. 4A,B); however, this worsening effect was not significant for the combination of eltoprazine 0.5 mg/kg plus amantadine 10 mg/kg, and the trend appeared to be caused by a single animal.

ON-time was significantly affected after both eltoprazine alone and the combined drugs (Fig. 4D), but this was counterbalanced by a trend toward increased



**FIG. 4.** (A–C) Time-course panels reporting parkinsonian disability clinical ratings (median) and locomotor activity counts (means) are shown without standard errors of the mean for readability. Administration of L-dopa, eltoprazine, or amantadine started at  $t=0$  minutes (\*, vs. vehicle [Veh] treatment; \$, vs. amantadine 10 mg/kg). Illustrated are (A) the impact of drug treatments on the parkinsonian disability time course accompanied by (B) the area under the curve (AUC) disability score, (C) the locomotor activity count time course, and (F) the AUCs. (D) ON-time was calculated based on the time during which the bradykinesia score was equal to 0. (E) Good ON-time (i.e., the on-time during which the dyskinesia score was equal to 0, 1, or 2) also is shown.

good ON-time observed with the combination of eltoprazine and amantadine (Fig. 4E). According to these observations, the locomotor activity counts were significantly reduced after both eltoprazine alone and the combined therapy (Fig. 4C,F). However, care should

be taken in interpreting locomotor activity counts, because they encompass both normal and abnormal movements.<sup>19,22</sup> Overall, the pattern was in favor of a significant reduction in LID with the combined drugs accompanied by a slight anti-L-dopa effect, but to a

lesser extent than the reduction produced with eltoprazine alone. Moreover, this resulted in increased good ON-time, a parameter easily detectable in clinical trials.

## Discussion

In the current study, we show that the mixed 5-HT<sub>1A/1B</sub> receptor agonist eltoprazine is highly effective in reducing LID in rat and monkey models of PD. However, this effect is accompanied by a partial loss of the therapeutic effect of L-dopa. Interestingly, our data also demonstrate that eltoprazine given at a low dose can be used to potentiate the antidyskinetic effect of amantadine. Albeit a reduction of the ON-time is still present after the combination, the disability score does not appear to be affected, suggesting that combination of the 2 drugs may represent a strategy to reduce the side effects and maintain significant antidyskinetic efficacy.

The partial 5-HT<sub>1A</sub> receptor agonist sarizotan has been investigated in clinical trials for its antidyskinetic effect. Although the results from the preclinical study and early, open-label clinical tests were promising, a larger double-blind clinical investigation was terminated for lack of efficacy. Although a detailed report has yet to be published, one reason of such failure may rely on the fact that activation of only 1 subtype of serotonin autoreceptors is not sufficient to provide significant antidyskinetic efficacy at doses devoid of side effects. In fact, our previous work indicated that significantly higher doses are required to produce the suppression of dyskinesia when targeting only the 5-HT<sub>1A</sub> receptors and that such doses are prone to induce side effects in rats, possibly due to the activation of postsynaptic 5-HT<sub>1A</sub> receptors.<sup>14</sup>

Eltoprazine, in contrast to sarizotan, can activate both the 5-HT<sub>1A</sub> and the 5-HT<sub>1B</sub> receptors, which allows the use of significant lower doses of drug because of the synergistic effect induced by simultaneous activation of both autoreceptor subtypes on silencing of serotonin neurons.<sup>3,4</sup> In addition, sarizotan is known to antagonize DA D<sub>2</sub> receptors, which also may contribute to the side effects observed in patients.<sup>10</sup> By contrast, eltoprazine is not known to have any antidopaminergic activity, which is supported here by the finding that the drug was not significantly effective against dyskinesia induced by apomorphine.

For these reasons, eltoprazine may have a significantly better chance of being clinically successful. In fact, eltoprazine is currently under clinical investigation, with promising preliminary results (see press release at [http://www.psychogenics.com/pdf/Positive\\_Efficacy\\_Data\\_in\\_Levodopa\\_Induced\\_Dyskinesia.pdf](http://www.psychogenics.com/pdf/Positive_Efficacy_Data_in_Levodopa_Induced_Dyskinesia.pdf)). Nevertheless, a partial anti-L-dopa effect was observed here and represents a concern. This could reflect a specific effect of

eltoprazine in the present animal models, but it also may be due to a general limitation of 5-HT<sub>1</sub> receptor activation. In fact, in subjects (both patients and parkinsonian animals) with advanced DA neurodegeneration, DA released from the serotonin neurons may represent the main source of L-dopa-derived DA. In this situation, the reduction of serotonin neuron activity obtained by treatment with 5-HT<sub>1</sub> receptor agonists may result not only in a reduction of dyskinesia but also in a partial loss of the therapeutic efficacy of L-dopa. The ongoing clinical trial will contribute to address this question. If partial worsening of the therapeutic effect of L-dopa is also observed in patients, then a combination of low doses of eltoprazine with amantadine may offer an alternative approach to reduce the appearance of side effects while increasing the antidyskinetic efficacy of the treatment. Thus, patients under amantadine treatment may be ideal candidates for recruitment into clinical studies investigating the antidyskinetic efficacy of eltoprazine.

**Acknowledgments:** Elisabetta Tronci was supported by Regione Autonoma della Sardegna (Project Master and Back, code PRR-MAB-A2011-19237). We thank Bengt Mattsson, Ulla Jarl, and Anneli Josefsson, for expert technical assistance.

## References

1. Sawada H, Oeda T, Kuno S, et al. Amantadine Study Group. Amantadine for dyskinesias in Parkinson's disease: a randomized controlled trial [serial online]. *PLoS One* 2010;31;5(12):e15298.
2. Meissner WG, Frasier M, Gasser T, et al. Priorities in Parkinson's disease research. *Nat Rev Drug Discov* 2011;10:377-393.
3. Carta M, Carlsson T, Kirik D, Bjorklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain* 2007;130:1819-1833.
4. Munoz A, Li Q, Gardoni F, et al. Combined 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor agonists for the treatment of L-DOPA-induced dyskinesia. *Brain* 2008;131:3380-3394.
5. Navailles S, Bioulac B, Gross C, De Deurwaerdere P. Chronic L-dopa therapy alters central serotonergic function and L-DOPA-induced dopamine release in a region-dependent manner in a rat model of Parkinson's disease. *Neurobiol Dis* 2011;41:585-590.
6. Bonifati V, Fabrizio E, Cipriani R, Vanacore N, Meco G. Buspirone in levodopa-induced dyskinesias. *Clin Neuropharmacol* 1994;17:73-82.
7. Goetz CG, Damier P, Hicking C, et al. Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. *Mov Disord* 2007;22:179-186.
8. Miczek KA, Mos J, Olivier B. Brain 5-HT and inhibition of aggressive behavior in animals: 5-HIAA and receptor subtypes. *Psychopharmacol Bull* 1989;25:399-403.
9. Mos J, Olivier B, Poth M, van Aken H. The effects of intraventricular administration of eltoprazine, 1-(3-trifluoromethylphenyl)piperazine hydrochloride and 8-hydroxy-2-(di-n-propylamino)tetralin on resident intruder aggression in the rat. *Eur J Pharmacol* 1992; 212:295-298.
10. Bartoszyk GD, Van Amsterdam C, Greiner HE, Rautenberg W, Russ H, Seyfried CA. Sarizotan, a serotonin 5-HT<sub>1A</sub> receptor agonist and dopamine receptor ligand. 1. Neurochemical profile. *J Neural Transm* 2004;111:113-126.
11. Peroutka SJ. Selective interaction of novel anxiolytics with 5-hydroxytryptamine<sub>1A</sub> receptors. *Biol Psychiatry* 1985;20:971-979.
12. Olivier B, Mos JM, Tulp M, Schipper J, Bevan P. Modulatory action of serotonin in aggressive behavior. In: Bevan P, Cools AR, Archer T, eds. *Behavioral Pharmacology of 5-HT*. Hillsdale, NJ: Lawrence Erlbaum Associates; 1989:89-115.



13. Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur J Neurosci* 2002;15:120–132.
14. Munoz A, Carlsson T, Tronci E, Kirik D, Bjorklund A, Carta M. Serotonin neuron-dependent and -independent reduction of dyskinesia by 5-HT1A and 5-HT1B receptor agonists in the rat Parkinson model. *Exp Neurol* 2009;219:298–307.
15. Schallert T, Tillerson J. Intervention strategies for degeneration of dopamine neurons in parkinsonism: optimising behavioral assessment of outcome. In: Emerich DF, Dean RL III, Sandberg PR, eds. *Innovative Models of CNS Disease: From Molecule to Therapy*. Clifton, NJ: Human Press; 1999:131–151.
16. Kirik D, Winkler C, Bjorklund A. Growth and functional efficacy of intrastriatal nigral transplants depend on the extent of nigrostriatal degeneration. *J Neurosci* 2001;21:2889–2896.
17. Ahmed MR, Berthet A, Bychkov E, et al. Lentiviral overexpression of GRK6 alleviates L-dopa-induced dyskinesia in experimental Parkinson's disease [serial online]. *Sci Transl Med* 2010;2:28ra28.
18. Bezard E, Dovero S, Prunier C, et al. Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *J Neurosci* 2001;21:6853–6861.
19. Bezard E, Ferry S, Mach U, et al. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. *Nat Med* 2003;9:762–767.
20. Fasano A, Daniele A, Albanese A. Early versus delayed bilateral subthalamic deep brain stimulation for Parkinson's disease: need for long-term clinical trials [letter]. *Mov Disord* 2011;26:1370; author reply 1371.
21. Guigoni C, Dovero S, Aubert I, et al. Levodopa-induced dyskinesia in MPTP-treated macaque is not dependent of the extent and pattern of the nigrostriatal lesion. *Eur J Neurosci* 2005;22:283–287.
22. Gold SJ, Hoang CV, Potts BW, et al. RGS9-2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. *J Neurosci* 2007;27:14338–14348.
23. Porras G, Berthet A, Dehay B, et al. PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. *J Clin Invest* 2012;22:3977–3989.
24. Fox SH, Johnston TH, Li Q, Brotchie J, Bezard E. A critique of available scales and presentation of the non-human primate dyskinesia rating scale. *Mov Disord* 2012;27:1373–1378.