British Journal of Pharmacology (1999) 128, 1577–1585
http://www.stockton-press.co.uk/bjp

The adrenergic receptor agonist, clonidine, potentiates the anti-parkinsonian action of the selective κ-opioid receptor agonist, enadoline, in the monoamine-depleted rat

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1 The treatment of Parkinson's disease relies predominantly upon dopamine replacement therapy, usually with l-dihydroxyphenylalanine (L-DOPA). However, side-effects of long-term treatment, such as L-DOPA-induced dyskinesias can be more debilitating than the disease itself. Non-dopaminergic treatment strategies might therefore be advantageous.

2 The aim of this study was to investigate the potential anti-parkinsonian efficacy of the κ-opioid receptor agonist, enadoline, and the α-adrenoreceptor agonist, clonidine, both alone or in combination, in the reserpine-treated rat model of Parkinson's disease.

3 Rats were treated with reserpine (3 mg kg^{-1}), and experiments carried out 18 h later, at which time they exhibited profound akinesia (normal animals 1251 ± 228 mobile counts h^{-1}, reserpine-treated animals 9 ± 2 mobile counts h^{-1}). Both enadoline and clonidine increased locomotion in reserpine-treated rats in a dose-dependent manner. The maximum locomotor-stimulating effect of enadoline alone was seen at a dose of 0.2 mg kg^{-1} (208 ± 63 mobile counts h^{-1}). The maximum effect of clonidine was seen at a dose of 2 mg kg^{-1} (536 ± 184 mobile counts h^{-1}).

4 Co-administration of enadoline (0.1 mg kg^{-1}) and clonidine (0.01–0.1 mg kg^{-1}) at sub-threshold doses, synergistically increased locomotion.

5 The synergistic stimulation of locomotion in the reserpine-treated rat involved activation of κ-opioid receptors and a combination of both α_1 and α_2-adrenoreceptors.

6 The results presented suggest a need for further studies on the potential of stimulating κ-opioid and/or α-adrenoreceptors as a therapy for Parkinson's disease. Furthermore, the studies may offer potential mechanistic explanations of the ability of α_2-adrenergic receptor antagonist to reduce L-DOPA-induced dyskinesia in Parkinson's disease.

Keywords: Parkinson's disease; κ-opioid; α-adrenoreceptor; reserpine; L-DOPA-induced dyskinesia

Abbreviations: L-DOPA, l-dihydroxyphenylalanine; L-threo-DOPS, L-threo-3,4-dihydroxyphenylserine; NMDA, N-methyl-D-aspartate; *nor*BNI, *nor*-Binaltorphimine

Introduction

Parkinson's disease is characterized by a loss of the dopamine-containing neurons in the nigrostriatal system. However, in addition to loss of dopaminergic neurons, there is a degeneration of other pigmented brainstem nuclei, in particular the locus coeruleus, which is a major source of ascending noradrenergic fibres (Greenfield & Bosanquet, 1953). Degeneration of the locus coeruleus leads to a decrease in the noradrenaline content of many forebrain areas including the substantia nigra, the caudate nucleus and the nucleus accumbens (Fahn *et al*., 1971; Rinne & Sonninen, 1973; Farley & Hornykiewicz, 1976). A number of studies suggest that decreased noradrenaline is important in the pathophysiology of Parkinson's disease. For instance, Narabayashi *et al*. (1981) demonstrated considerable benefit from the administration of L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS), an immediate precursor of noradrenaline, to Parkinson's disease patients. In addition, the reversal of reserpine-induced akinesia by l-dihydroxyphenylalanine (L-DOPA) is associated with an increase in brain noradrenaline levels, as well as dopamine levels (Dolphin *et al*., 1976a), and can be inhibited by administration of adrenoreceptor antagonists or inhibitors of dopamine β-hydroxylase, an enzyme that converts dopamine to noradrenaline in noradrenergic neurons (Dolphin *et al*., 1976b). The inhibitory effect of dopamine β-hydroxylase inhibitors on L-DOPA-induced reversal of akinesia induced by reserpine, can be reversed by administration of clonidine, an α-adrenoreceptor agonist (Dolphin *et al*., 1976c). In addition, stimulation of α-adrenoreceptors with clonidine enhances the anti-parkinsonian actions in combination with dopamine receptor agonists (Anden *et al*., 1973; Anden & Strombom, 1974; Dolphin *et al*., 1976b; Pycock *et al*., 1977; Eshel *et al*., 1990) and muscarinic receptor antagonists (Carlsson & Carlsson, 1989b; Carlsson *et al*., 1991; Gomez-Mancilla *et al*., 1991). Together, these studies suggest that restoration of noradrenergic function at α-adrenoreceptors may be of value in the treatment of Parkinson's disease.

Furthermore, glutamatergic neurotransmission in several regions of the basal ganglia is overactive in Parkinson's disease and blockade of this enhanced glutamate transmission has anti-parkinsonian actions (Miller & Delong, 1987; Albin *et al*., 1989; Brotchie *et al*., 1991; 1993; Brotchie 1997; Carroll *et al*., 1995; Mitchell *et al*., 1995; Kaur & Starr, 1997). Stimulation of α-adrenoreceptors potentiates the anti-parkinsonian effects of glutamate receptor antagonists (Carlsson & Carlsson, 1989a; 1990; Carlsson & Svensson, 1990).

We have demonstrated that κ-opioid receptor agonists can inhibit the release of glutamate in these overactive areas of the

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basal ganglia (Hill and Brotchie, 1995; 1999; Maneuf *et al*., 1995), and we have shown that systemic administration of κ-opioid agonists has anti-parkinsonian properties in rat (Hughes *et al*., 1998) and marmoset (Mitchell *et al*., 1995) models of Parkinson's disease. With this aim, we tested the hypothesis that stimulation of α-adrenergic receptors will enhance the anti-parkinsonian actions of enadoline and characterized the pharmacology of such effects.

In the present study, the effect of co-administration of enadoline and clonidine was investigated in the reserpine-treated rat model of Parkinson's disease.

Methods

Reserpine-treated rat model of Parkinson's disease

Male Sprague Dawley rats (200–250 g) were injected subcutaneously (s.c.) with 3 mg kg^{-1} reserpine (dissolved in 1% glacial acetic acid in distilled water) or vehicle (1 ml kg^{-1}) under light inhalation anaesthesia (Halothane). Experiments were carried out 18 h later, as described below.

Assessment of locomotor activity in systemically treated rats

Animals were acclimatized in the experimental room for at least 30 min before beginning experiments. All drugs were administered i.p. (concentrations and times as defined in Figure legends). The locomotion of animals was assessed using an automated movement detection system (Benwick Data-logger, Linton Instrumentation, U.K.), which consisted of a frame (52 × 36 cm) containing a series of infrared red beams situated along the exterior of a frame. Animals were isolated from the frame by a perspex box placed inside the frame. The movement of animals was detected when the animal passed from one grid to another and so broke an infrared beam. Locomotor activity was monitored using Amlogger software (Linton Instrumentation, U.K.), which logged data at 5 min intervals over a period of 60 min. The index of locomotion used in the studies was mobile counts, which are recorded when the central position of the animal changes by more than two grids in any one second period. Experimental data was analysed using a spreadsheet program (Microsoft Excel). For analysis, mobile counts were taken as a total over a 60 min period or were plotted as a timecourse with 5 min intervals. In addition, a subjective behavioural assessment of the animals was also noted, although no analysis was performed on this data.

Statistical analyses

Gaussian distributions of all parameters were assumed throughout all experiments. Differences between means were compared by a one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Timecourse data was analysed with a two-way ANOVA followed by a bonferroni post-test. The level of significance for rejection of the null hypothesis was set at $P<0.05$.

Materials

Clonidine, prazosin and reserpine were purchased from Sigma (U.K.). *nor*-Binaltorphimine (*nor*-BNI) and rauwolscine were purchased from RBI (U.K.). Enadoline was a generous gift from Parke-Davis Neuroscience Centre (Cambridge, U.K.).

Results

Effect of reserpine on locomotor activity in the rat

Locomotor activity in non-reserpinized, vehicle-treated rats was 1251 ± 228 mobile counts h^{-1}. Following administration of reserpine (3 mg kg^{-1}), locomotor activity was less than 1% of that recorded in non-reserpinized vehicle treated rats (9 ± 2 mobile counts h^{-1}, $P<0.001$, Figure 1).

Effect of enadoline on locomotion in the reserpine-treated rat

Enadoline increased locomotion in reserpine-treated rats in a dose-dependent manner (one-way ANOVA, $F_{3,27}=15.39$, $P<0.0001$, Figure 1a). The maximum effect was seen at 0.2 mg kg^{-1} enadoline (208 ± 63 mobile counts h^{-1}) and was significantly higher than vehicle-treated rats ($P<0.001$; one-way ANOVA, followed by Tukey-Kramer multiple comparisons test). Figure 1b shows the timecourse of the effect of

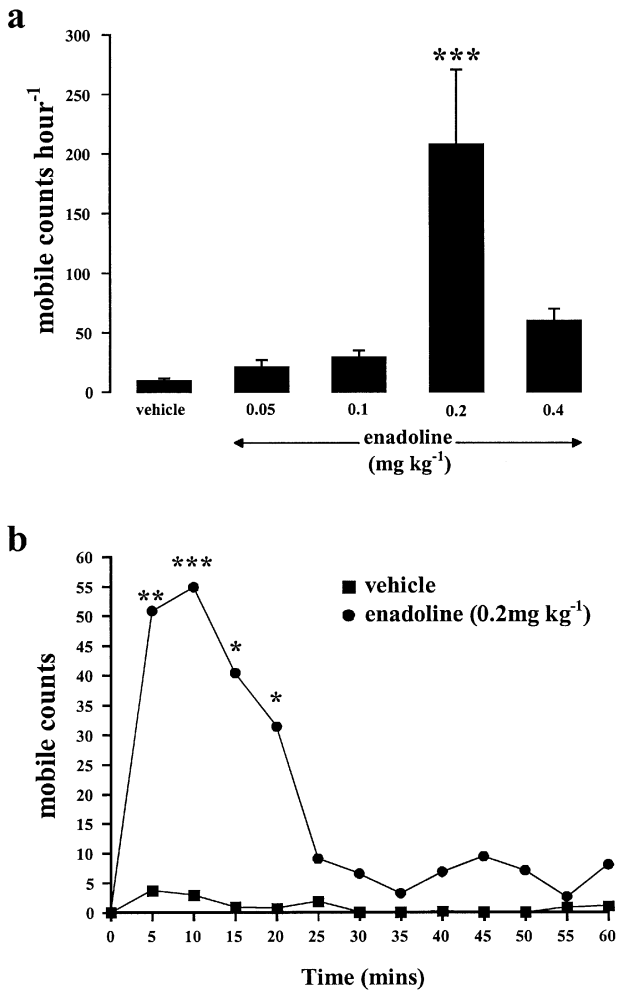

Figure 1 Enadoline stimulates locomotion in the reserpine-treated rat. Enadoline (0.05–0.4 mg kg^{-1}) or vehicle (1 ml kg^{-1}) was administered i.p. and locomotion monitored for 60 min. (a) Dose-response of effect of enadoline (0.05–0.4 mg kg^{-1}) on locomotion and (b) timecourse of effect of enadoline (0.2 mg kg^{-1}) on locomotion. Values are expressed as (a) mean ± s.e.mean, $^{***}P<0.001$ compared to vehicle, $n=5$–11 animals (one-way ANOVA, followed by Tukey-Kramer multiple comparisons test) and (b) mean, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to vehicle, $n=6$–11 animals (two-way ANOVA, followed by bonferroni post-test; error bars are omitted for clarity).

0.2 mg kg^{-1} enadoline. Enadoline (0.2 mg kg^{-1}) increased locomotion with a short latency (less than 5 min) and a peak effect at 10 min post-injection ($P<0.001$, two-way ANOVA, followed by bonferroni post-test, Figure 1b). The quality of locomotion in reserpine-treated rats that received enadoline was not as fluid as that observed in non-reserpinized, vehicle-treated rats. Furthermore, periods of retroversive locomotion were observed. At the two highest doses used (0.2 and 0.4 mg kg^{-1}), enadoline also had a pronounced diuretic effect. At a dose of 0.4 mg kg^{-1} enadoline the rats were noticeably sedated.

Following co-administration of *nor*-BNI (3 mg kg^{-1}) and enadoline, locomotion in the reserpine-treated rat was found to be 53±16 mobile counts h^{-1}. This was 75±6% less than that seen following enadoline alone ($P<0.001$, one-way ANOVA followed by Tukey-Kramer multiple comparisons test (Figure 2). Administration of *nor*-BNI (3 mg kg^{-1}) alone, had no effect on locomotion in the reserpine-treated rat (Table 1).

Effect of clonidine on locomotion in the reserpine-treated rat

Clonidine increased locomotion in reserpine-treated rats in a dose-dependent manner (one-way ANOVA, $F_{6,34}=11.37$, $P<0.0001$, Figure 3a). The maximum effect was seen at a dose of 2 mg kg^{-1} clonidine (536±184 mobile counts h^{-1}) and was significantly higher than vehicle-treated rats ($P<0.0001$; one-way ANOVA, followed by Tukey-Kramer multiple comparisons test). Following injection of a dose of 2 mg kg^{-1} clonidine, there was a steady increase in locomotion post-injection, with a peak effect at 55 min post-injection ($P<0.001$, two-way ANOVA, followed by bonferroni post-test; Figure 3b).

As with enadoline alone, the quality of locomotion in reserpine-treated rats that received clonidine alone was not comparable to that observed in non-reserpinized, vehicle-treated rats. At higher doses (1 and 2 mg kg^{-1}) periods of wall climbing behaviour and vertical jumping were observed. Furthermore, on some occasions rats exhibited signs of stereotyped behaviour where they continually 'marched' around the edge of the perspex box. At all the doses of clonidine used, the rats exhibited piloerection and mydriasis.

Rauwolscine (1 mg kg^{-1} i.p.) and prazosin (1 mg kg^{-1} i.p.) reduced clonidine-induced locomotion by 70±8% and

Table 1 The effect of *nor*-BNI, rauwolscine and prazosin on locomotion in the reserpine-treated rat

Treatment	*Mobile counts h^{-1}*
Vehicle	9.3±2.1
nor-BNI (3 mg kg^{-1})	11.7±4.8
Rauwolscine (1 mg kg^{-1})	5.0±3.6
Rauwolscine (3 mg kg^{-1})	15.4±9.7
Rauwolscine (10 mg kg^{-1})	57.0±14.2*
Prazosin (1 mg kg^{-1})	20.8±12.7
Prazosin (3 mg kg^{-1})	14.1±5.8

Values are expressed as the mean±s.e.mean; *$P<0.05$ compared to vehicle treated rats; $n=6-12$ (one-way ANOVA, followed by Tukey-Kramer multiple comparisons test).

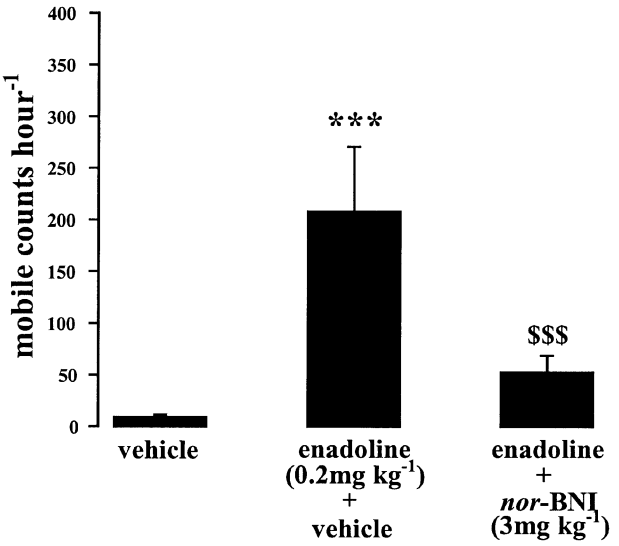

Figure 2 *nor*-BNI inhibits enadoline-induced stimulation of locomotion in the reserpine-treated rat. *nor*-BNI (3 mg kg^{-1} i.p.) was administered 90 min prior to enadoline (0.2 mg kg^{-1}) or vehicle (1 ml kg^{-1}) and locomotion monitored for 60 min. Values are expressed as mean±s.e.mean, ***$P<0.001$ compared to vehicle; $$$$P<0.001$ compared to enadoline, $n=5-8$ animals (one-way ANOVA followed by Tukey-Kramer multiple comparisons test).

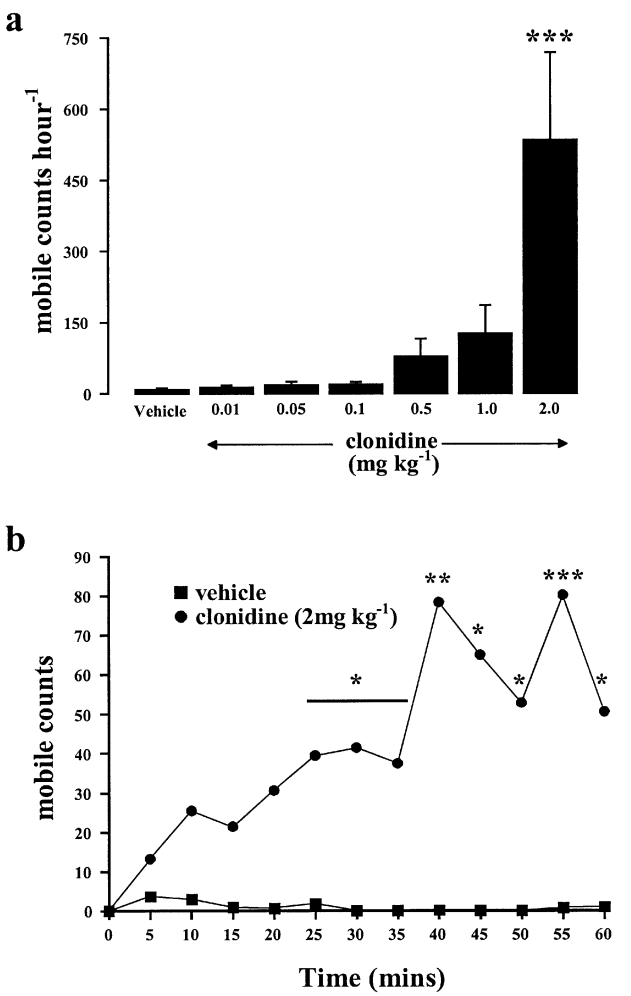

Figure 3 Clonidine stimulates locomotion in the reserpine-treated rat. Clonidine (0.01–2 mg kg^{-1}) or vehicle (1 ml kg^{-1}) was administered i.p. and locomotion monitored for 60 min. (a) Dose-response of effect of clonidine (0.01–2 mg kg^{-1}) on locomotion and (b) timecourse of effect of clonidine (2 mg kg^{-1}) on locomotion. Values are expressed as (a) mean±s.e.mean, ***$P<0.001$ compared to vehicle, $n=4-10$ animals (one-way ANOVA, followed by Tukey-Kramer multiple comparisons test) and (b) mean *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to vehicle, $n=6-10$ animals (two-way ANOVA, followed by bonferroni post-test; error bars are omitted for clarity).

84±11%, respectively ($P<0.05$ for both, one-way ANOVA followed by Tukey-Kramer multiple comparisons test, Figure 4). Administration of combination of rauwolscine (1 mg kg^{-1} i.p.) and prazosin (1 mg kg^{-1} i.p.) reduced clonidine-induced locomotion by 89±9% ($P<0.01$ one-way ANOVA followed by Tukey-Kramer multiple comparisons test, Figure 4). Administration of rauwolscine (1–3 mg kg^{-1} i.p.) or prazosin (1–3 mg kg^{-1} i.p.) alone, had no effect on locomotion in the reserpine-treated rat. However, rauwolscine (10 mg kg^{-1}) significantly increased locomotion (Table 1).

Effect of combined enadoline and clonidine treatment on locomotion in the reserpine-treated rat

Clonidine (0.01– 0.1 mg kg^{-1}) potentiated the locomotor stimulant effect of enadoline (0.1 mg kg^{-1}) in a dose-dependent manner (one-way ANOVA, $F_{4,33}=12.68$, $P<0.0001$, Figure 5a). The maximum effect was seen at 0.05 mg kg^{-1} clonidine (228±54 mobile counts h^{-1}) and was significantly higher than in rats treated with vehicle ($P<0.001$; one-way ANOVA, followed by Tukey-Kramer multiple comparisons test) or enadoline alone ($P<0.001$; one-way ANOVA, followed by Tukey-Kramer multiple comparisons test). The combined enadoline plus clonidine (0.05 mg kg^{-1}) treatment increased locomotion in the reserpine-treated rat in a biphasic manner with peaks of activity at approximately 20 min ($P<0.05$, two-way ANOVA, followed by bonferroni post-test) and 45 min ($P<0.001$, two-way ANOVA, followed by bonferroni post-test) post injection (Figure 5b).

The quality of locomotion in reserpine-treated rats that received enadoline plus clonidine was not as good as that observed in non-reserpinized vehicle-treated rats. In some rats grooming and sniffing behaviour was observed. At the highest dose of clonidine (0.1 mg kg^{-1}) plus enadoline (0.1 mg kg^{-1}) the rats were noticeably sedated. Periods of wall climbing behaviour and vertical jumping were also observed at this highest dose. In some animals, retroversive locomotion, mydriasis, diuresis and piloerection was also observed. Furthermore, on some occasions, rats exhibited signs of stereotyped behaviour where they continually 'marched' around the edge of the perspex box.

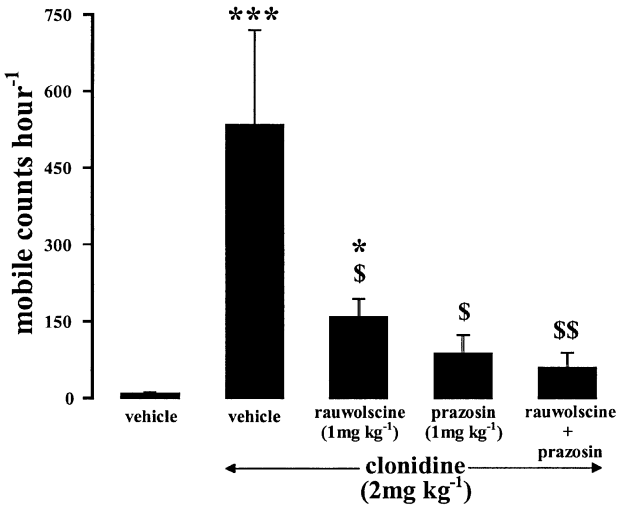

Figure 4 Rauwolscine and prazosin inhibit clonidine-induced stimulation of locomotion in the reserpine-treated rat. Rauwolscine (1 mg kg^{-1} i.p.) and prazosin (1 mg kg^{-1} i.p.) were administered 30 min prior to clonidine (2 mg kg^{-1}) or vehicle (1 ml kg^{-1}) and locomotion was then monitored for 60 min. Values are expressed as mean±s.e.mean, $*P<0.05$, $***P<0.001$, compared to vehicle; $\$P<0.05$, $\$\$P<0.01$, compared to clonidine 5–6 animals (one-way ANOVA, followed by Tukey-Kramer multiple comparisons test).

Effect of nor-BNI, rauwolscine and prazosin on combined enadoline and clonidine-induced locomotion in the reserpine-treated rat

Following administration of *nor*-BNI (3 mg kg^{-1} i.p.) the first phase (10–25 min post injection) and second phase (35–50 min post injection) of cumulated enadoline plus clonidine (0.05 mg kg^{-1})-induced locomotion in the reserpine-treated rat were both reduced (78±7 and 84±14%, respectively; both $P<0.001$, one-way ANOVA followed by Tukey-Kramer multiple comparisons test, Figure 6a, b).

Rauwolscine (1, 3 and 10 mg kg^{-1}) had no effect on the first phase (10–25 min post injection, Figure 6a). However, the second phase (35–50 min post injection) was significantly

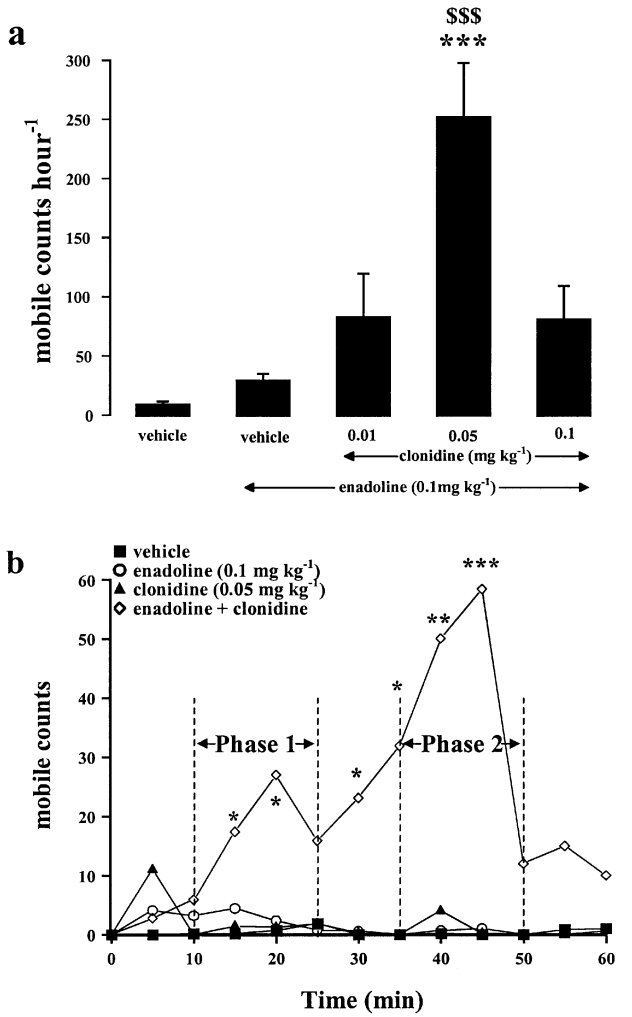

Figure 5 Clonidine and enadoline synergistically stimulate locomotion in the reserpine-treated rat. Clonidine (0.01–0.1 mg kg^{-1} i.p.), enadoline (0.1 mg kg^{-1}) or vehicle (1 ml kg^{-1}) were administered i.p. and locomotion monitored for 60 min. (a) Effect of clonidine (0.01–0.1 mg kg^{-1}) plus enadoline (0.1 mg kg^{-1}) on locomotion. (b) Timecourse of the effect of clonidine (0.05 mg kg^{-1}) plus enadoline (0.1 mg kg^{-1}) on locomotion. Values are expressed as (a) mean± s.e.mean, $***P<0.001$ compared to vehicle, $\$\$\$P<0.001$ compared to enadoline alone $n=4$–11 animals (one-way ANOVA, followed by Tukey-Kramer multiple comparisons test) and (b) mean, $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to enadoline alone, $n=6$–11 animals (two-way ANOVA, followed by bonferroni post-test; error bars are omitted for clarity).

reduced, by 76±8, 81±10 and 74±9%, following co-administration of rauwolscine (1, 3 and 10 mg kg^{-1}, respectively) compared to enadoline plus clonidine alone (all $P<0.001$, one-way ANOVA followed by Tukey-Kramer multiple comparisons test, Figure 6b).

In contrast, prazosin (1 and 3 mg kg^{-1}) reduced the first phase (10–25 min post injection) of enadoline plus clonidine-induced locomotion by 87±5 and 81±7%, respectively (both $P<0.001$, one-way ANOVA, followed by Tukey-Kramer multiple comparisons test, Figure 6a). Following administra-

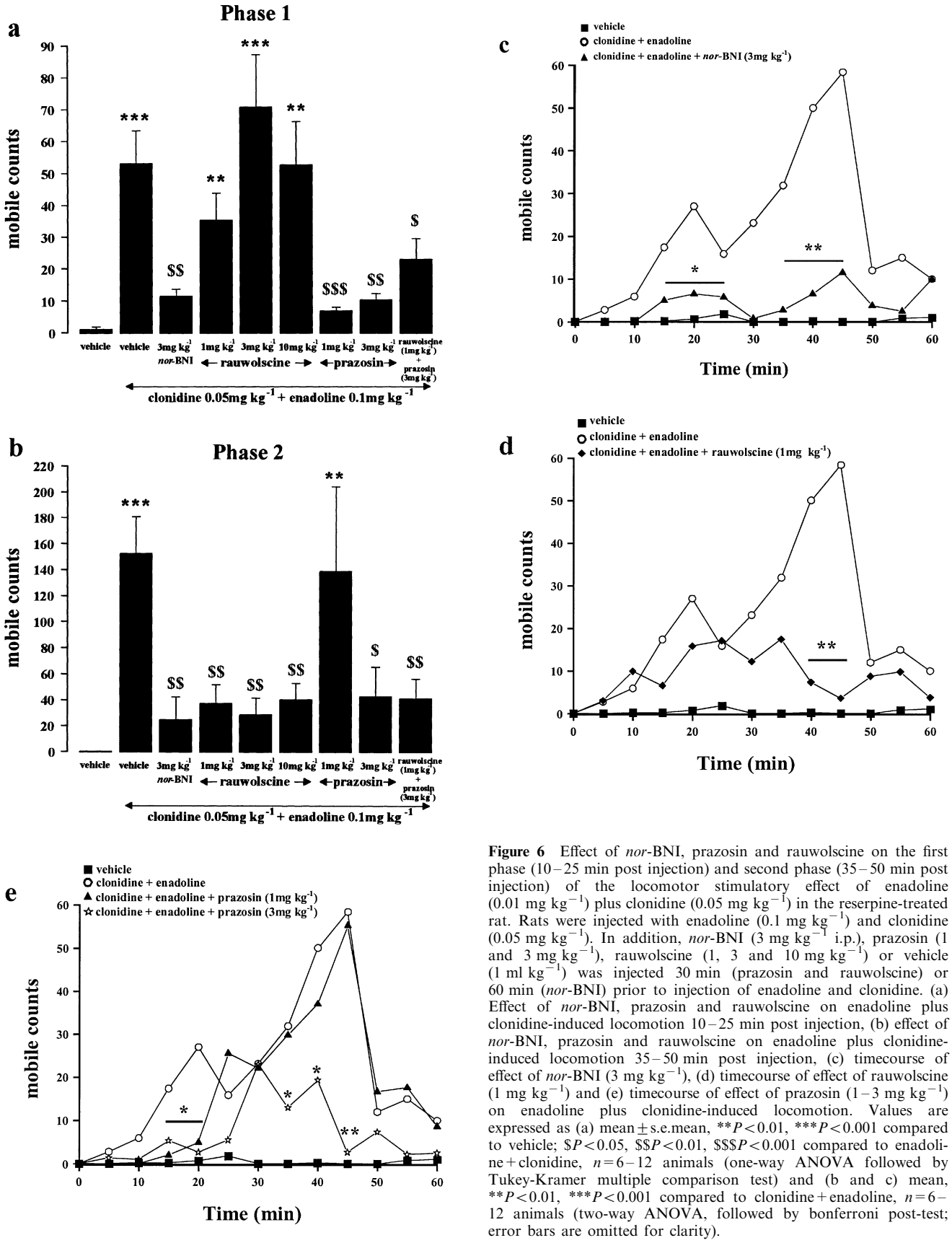

Figure 6 Effect of *nor*-BNI, prazosin and rauwolscine on the first phase (10–25 min post injection) and second phase (35–50 min post injection) of the locomotor stimulatory effect of enadoline (0.01 mg kg^{-1}) plus clonidine (0.05 mg kg^{-1}) in the reserpine-treated rat. Rats were injected with enadoline (0.1 mg kg^{-1}) and clonidine (0.05 mg kg^{-1}). In addition, *nor*-BNI (3 mg kg^{-1} i.p.), prazosin (1 and 3 mg kg^{-1}), rauwolscine (1, 3 and 10 mg kg^{-1}) or vehicle (1 ml kg^{-1}) was injected 30 min (prazosin and rauwolscine) or 60 min (*nor*-BNI) prior to injection of enadoline and clonidine. (a) Effect of *nor*-BNI, prazosin and rauwolscine on enadoline plus clonidine-induced locomotion 10–25 min post injection, (b) effect of *nor*-BNI, prazosin and rauwolscine on enadoline plus clonidine-induced locomotion 35–50 min post injection, (c) timecourse of effect of *nor*-BNI (3 mg kg^{-1}), (d) timecourse of effect of rauwolscine (1 mg kg^{-1}) and (e) timecourse of effect of prazosin (1–3 mg kg^{-1}) on enadoline plus clonidine-induced locomotion. Values are expressed as (a) mean±s.e.mean, $^{**}P<0.01$, $^{***}P<0.001$ compared to vehicle; $\$P<0.05$, $\$\$P<0.01$, $\$\$\$P<0.001$ compared to enadoline+clonidine, $n=6$–12 animals (one-way ANOVA followed by Tukey-Kramer multiple comparison test) and (b and c) mean, $^{**}P<0.01$, $^{***}P<0.001$ compared to clonidine+enadoline, $n=6$–12 animals (two-way ANOVA, followed by bonferroni post-test; error bars are omitted for clarity).

tion of prazosin (3 mg kg^{-1}) the second phase (35–50 min post injection) of enadoline plus clonidine-induced locomotion was reduced by 72±15% compared to enadoline plus clonidine alone ($P<0.01$; one-way ANOVA, followed by Tukey-Kramer multiple comparisons test, Figure 6b). However, the lower dose of prazosin (1 mg kg^{-1}) had no effect on the second phase of locomotor stimulation.

Discussion

As previously described on many occasions, administration of reserpine to rats produces some symptoms similar to those seen in the parkinsonian patient, i.e. akinesia and muscular rigidity (Colpaert, 1987; Lorenc-Koci *et al*., 1995). Furthermore, drugs which are effective anti-parkinsonian agents are effective in restoring locomotor function in the reserpine-treated rat (Colpaert, 1987; Ushijima *et al*., 1988; Carlsson *et al*., 1991; Ferre *et al*., 1991a, b). In the present study, administration of reserpine reduced locomotion by over 99% compared to non-reserpinized, vehicle-treated rats (Figure 1).

Effect of enadoline on locomotion in the reserpine-treated rat model of Parkinson's disease

Systemic administration of enadoline to reserpine-treated rats caused a dose-dependent increase in locomotion (Figure 1a) with a maximal effect at 0.2 mg kg^{-1}. This effect of κ-opioid receptor stimulation on locomotion has been reported previously (McDougall *et al*., 1997; Hughes *et al*., 1998). The effect of enadoline was mediated by κ-opioid receptor activation as it was completely abolished by prior administration of the selective κ-opioid receptor antagonist *nor*-BNI (Portoghese *et al*., 1987) (Figure 2). At the dose used, *nor*-BNI has been shown to have effects that are selective for κ-opioid receptors (Endoh *et al*., 1992). At high doses of enadoline, sedative (0.4 mg kg^{-1}) and diuretic effects (0.2 and 0.4 mg kg^{-1}) were apparent. This is consistent with the expected profile of a κ-opioid receptor agonist (Leander, 1983; Ukai & Kameyama, 1985; Leighton *et al*., 1987; Dykstra *et al*., 1987; Peters *et al*., 1987; Hunter *et al*., 1990). The sedative effect at higher doses probably underlies the bell-shaped nature of the dose response curve for locomotor stimulation by enadoline (Figure 1a). Administration of the tyrosine hydroxylase inhibitor, α-methyl-*para*-tyrosine, 90 min prior to injection of enadoline, had no effect on the locomotor stimulant effect of enadoline, suggesting that the effect of enadoline is not mediated through an effect on residual dopamine transmission (data not shown). In fact, activation of striatal κ-opioid receptors has previously been shown to inhibit dopamine release in the rat striatum (Di Chiara & Imperato, 1988; Spanagel *et al*., 1990a, b; Longoni *et al*., 1991). κ-Opioid receptor agonists have been shown to have motor-activating effects when injected into the output regions of the basal ganglia, i.e. the substantia nigra, medial segment of the globus pallidus (in the primate) or entopeduncular nucleus (in the rat) (Maneuf *et al*., 1995; Thompson & Walker, 1992; Matsumoto *et al*., 1988a, b; Morelli & Di Chiara, 1985; Herrera-Marschitz *et al*., 1983; 1984; 1986; Von Voightlander *et al*., 1983). Therefore, the locomotor-stimulant effect of systemic enadoline in the present study may in part be due to effects in the output regions of the basal ganglia. However, we have also shown that activation of κ-opioid receptors in the striatum inhibits the release of glutamate from striatal nerve terminals (Hill & Brotchie, 1995; 1999) and that blockade of striatal N-methyl-D-aspartate (NMDA) subtype of glutamate receptors has anti-parkinsonian actions (Carroll *et al*., 1995; Mitchell *et al*., 1995; Kaur & Starr, 1997; Nash *et al*., 1999). Therefore, the locomotor activating effects of enadoline might also be due to an effect on glutamate transmission in the striatum. However, the narrow dose range at which the effects of enadoline are seen suggest that enadoline alone is unlikely to have any therapeutic benefit in the treatment of Parkinson's disease.

Effect of clonidine on locomotion in the reserpine-treated rat model of Parkinson's disease

Systemic administration of clonidine to reserpine-treated rats elicited a dose-dependent increase in locomotion (Figure 3a) with a maximal effect at 2 mg kg^{-1}. This is in agreement with a previous study that demonstrated an anti-cataleptic effect of clonidine in the reserpine-treated rat (Maj *et al*., 1993). Furthermore, clonidine inhibits the catalepsy produced by haloperidol or fluphenazine (Wielosz *et al*., 1978). Previous studies have also demonstrated that administration of clonidine can induce catalepsy and reduce locomotor activity in mice (Pranzatelli *et al*., 1987; Sukul *et al*., 1988) and has no effect in rats (Wagner & Anderson, 1982). This is in contrast to the results presented here where clonidine increased locomotion. However, these differences almost certainly reflect the use of different animal models (i.e. not monoamine depleted) than the one used in the present studies. The effect of clonidine was mediated by activation of α_1- and α_2-adrenoceptors as either rauwolscine (1 mg kg^{-1}) or prazosin (1 mg kg^{-1}) blocked the locomotor stimulant effect of clonidine (Figure 4). This is somewhat surprising as clonidine is generally thought of as a selective α_2-adrenoreceptor antagonist. However, a previous study demonstrated that clonidine potentiated the locomotor stimulant effect of the selective D_2-dopamine receptor agonist quinpirole in monoamine-depleted mice, *via* an action of α_1-adrenoreceptors (Eshel *et al*., 1990). Administration of a combination of rauwolscine and prazosin (1 mg kg^{-1} i.p.) reduced clonidine-induced locomotion to an extent that was not significantly different to either alone (Figure 4). This suggests a synergistic effect of clonidine acting at both α_1- and α_2-adrenoreceptors. Administration of the tyrosine hydroxylase inhibitor, α-methyl-*para*-tyrosine, 90 min prior to injection of clonidine had no effect on the locomotor stimulant effect of clonidine (data not shown), suggesting that the effect of clonidine is not mediated through an effect on dopamine transmission. In rodent brain, the distribution of α_2-adrenoreceptor subtypes varies between different regions. α_{2a}-Adrenoreceptor mRNA is predominantly found in the cerebral cortex, locus coeruleus, hippocampus and cerebellum (Zeng & Lynch, 1991; McCune *et al*., 1993; Nicholas *et al*., 1993; MacDonald & Scheinin, 1995). α_{2b}-Adrenoreceptors are relatively scarce but are found in low quantities in the thalamus (Zeng & Lynch, 1991; Nicholas *et al*., 1993; MacDonald & Scheinin, 1995). α_{2c}-Adrenoreceptors are found in a number of brain regions but the highest density is in the basal ganglia (Zeng & Lynch, 1991; Nicholas *et al*., 1993; MacDonald & Scheinin, 1995). α_1-Adrenergic receptor mRNA is widely distributed within the rat CNS, however it is predominantly found in the cerebral cortex, thalamus, dorsal raphe nucleus and the pineal gland. However, there is very little found within the basal ganglia (Nicholas *et al*., 1996). Further studies using intracerebral administration of clonidine will be required to determine the site of action of its motor stimulating effects in the reserpine-treated rat.

Effect of co-administration of enadoline and clonidine on locomotion in the reserpine-treated rat model of Parkinson's disease

Systemic administration of a sub-threshold dose of enadoline (0.1 mg kg^{-1}) plus clonidine (0.01 – 0.1 mg kg^{-1}) to reserpine-treated rats elicited an increase in locomotion with a maximal effect at 0.05 mg kg^{-1} clonidine (Figure 5a). At higher doses of clonidine, the locomotor stimulant effect was reduced, possibly due to the appearance of sedative side-effects. Previous studies have demonstrated that clonidine also potentiates the locomotor-stimulant effects of competitive or non-competitive NMDA receptor antagonists (Carlsson & Carlsson, 1989a; 1990; Carlsson & Svensson, 1990), dopamine receptor agonists (Anden *et al*., 1973; Anden & Strombom, 1974; Dolphin *et al*., 1976b; Pycock *et al*., 1977; Eshel *et al*., 1990) and muscarinic receptor antagonists (Carlsson & Carlsson, 1989b; Carlsson *et al*., 1991; Gomez-Mancilla *et al*., 1991).

The timecourse of the effect of enadoline (0.1 mg kg^{-1}) plus clonidine (0.05 mg kg^{-1}) was biphasic with peaks of activity at approximately 20 and 45 min post-injection (Figure 5b). This is in contrast to the monophasic timecourse of effective doses of enadoline (Figure 1b) and clonidine (Figure 3b) when administered alone. The first phase (10 – 25 min post injection) of activity was inhibited by *nor*-BNI and prazosin (Figure 6a), suggesting a κ-opioid receptor and α_1-adrenoreceptor-dependent mechanism. The second phase (35 – 50 min post injection) of activity was inhibited by *nor*-BNI and rauwolscine (Figure 6b), suggesting that the second phase is dependent on κ-opioid and α_2-adrenoreceptor activation. It is possible that rauwolscine and prazosin are blocking the κ-opioid receptor to have their effects in this model. However, the κ-opioid receptor-antagonist *nor*-BNI blocked both phases of the response to enadoline + clonidine, whereas prazosin or rauwolscine only blocked the first or second phase, respectively. Therefore, prazosin and rauwolscine have different profiles in this model than *nor*-BNI, which suggests they are not acting at the κ-opioid receptor at the doses used. Of interest was the finding that a high dose of prazosin (3 mg kg^{-1}) blocked a substantial portion of the second phase while the low dose of prazosin (1 mg kg^{-1}) blocked only the first phase. This suggests that either α_1-adrenoreceptors with a lower affinity for prazosin are involved in the second phase or that the higher dose of prazosin is blocking a different receptor. The first scenario is unlikely as prazosin has been shown to have no selectivity between α_{1a}-, α_{1b}- or α_{1c}-adrenoreceptors (Kenny *et al*., 1994). Furthermore, as rauwolscine did not have an additive inhibitory effect with the high dose of prazosin, an α_2-adrenoreceptor-dependent mechanism is implicated. An alternative explanation is that prazosin is blocking α_2-adrenoreceptors at the dose used. Indeed, prazosin has been shown to have α_2-adrenoreceptor antagonist activity, with higher affinity for $\alpha_{2b/c}$-adrenoreceptors compared to α_{2a}-adrenoreceptors (Blaxall *et al*., 1991; Harrison *et al*., 1991; Lanier *et al*., 1991; Bylund *et al*., 1992). Interestingly, rauwolscine has also been reported to be more selective for α_{2c}-adrenoreceptors (Blaxall *et al*., 1991; Harrison *et al*., 1991; Bylund *et al*., 1992). As described above, α_{2c}-adrenoreceptors are synthesized by striatal neurons (Zeng & Lynch, 1991; Nicholas *et al*., 1993; MacDonald & Scheinin, 1995). Given the likely involvement of the striatopallidal/nigral system in the anti-parkinsonian actions of enadoline it is attractive to speculate that basal ganglia α_{2c}-adrenoreceptor activation may indeed be responsible for the clonidine-induced enhancement of the locomotor stimulant action of enadoline. However, more selective antagonists at α_2-adrenoreceptor subtypes and intracerebral injection studies will be needed to confirm this hypothesis.

The results presented here demonstrate that enadoline and clonidine have locomotor-stimulant effects in the monoamine-depleted rat. Furthermore, in combination, there is a pronounced synergistic stimulation of locomotion. This synergistic stimulation of locomotion involved activation of κ-opioid receptors and a combination of α_1 and α_2-adrenoreceptors.

Further work will be required to establish whether κ-opioid plus/or α-adrenoreceptor stimulation will be useful as an anti-parkinsonian therapy. It must be noted that the narrow dose range at which enadoline has locomotor-stimulant effects when administered alone, may preclude its use in the treatment of Parkinson's disease. Furthermore, the quality of movement seen following enadoline or clonidine was not as good as that which is seen following dopamine agonist therapy. Indeed, we and others have previously suggested a role for stimulation of α-adrenoreceptors in the genesis of L-DOPA-induced dyskinesia (Brefel-Courbon *et al*., 1998; Brotchie, 1998; Henry *et al*., 1998). Studies in primates, where it is possible to distinguish stimulation of locomotion from dyskinesia, will be necessary to determine the clinical applicability of the findings reported here. However, enadoline and clonidine therapy in low doses may be useful as an adjunct to current dopamine-replacement therapy in Parkinson's disease. Indeed, we have previously demonstrated that enadoline potentiates the locomotor activating effects of L-DOPA in the reserpine-treated rat (Hughes *et al*., 1998).

We are grateful to the Parkinson's Disease Society for funding the research and to Dr A.M. McKnight at the Parke-Davis Neuroscience Centre (Cambridge, U.K.) for the generous gift of enadoline.

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(Received July 12, 1999
Accepted September 15, 1999)