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# Locomotor effects of imidazoline $I_2$-site-specific ligands and monoamine oxidase inhibitors in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway

[1]Nicholas MacInnes & *,[1]Susan Duty

[1]Wolfson Centre for Age-Related Diseases, G20 Wolfson Wing, Hodgkin Building, Guy's Campus, GKT School of Biomedical Sciences, King's College London, London SE1 1UL

**1** The present study examined the ability of the selective imidazoline $I_2$-site ligands 2-(-2-benzofuranyl)-2-imidazoline (2-BFI) and 2-[4,5-dihydroimidaz-2-yl]-quinoline (BU224) and selected monoamine oxidase (MAO) inhibitors to evoke locomotor activity in rats bearing a lesion of the nigrostriatal pathway.

**2** Male Sprague–Dawley rats were injected with 12.5 $\mu$g 6-hydroxydopamine (6-OHDA) into the right median forebrain bundle to induce a unilateral lesion of the nigrostriatal tract. After 6 weeks, test drugs were administered either alone or in combination with L-DOPA (L-3,4-dihydroxyphenylamine) and the circling behaviour of animals was monitored as an index of anti-Parkinsonian activity.

**3** Intraperitoneal (i.p.) administration of the irreversible MAO-B inhibitor deprenyl (20 mg kg$^{-1}$) or the imidazoline $I_2$-site ligands BU224 (14 mg kg$^{-1}$) and 2-BFI (7 and 14 mg kg$^{-1}$) produced significant increases in ipsiversive rotations compared to vehicle controls totaling, at the highest respective doses tested, 521 $\pm$ 120, 131 $\pm$ 37 and 92.5 $\pm$ 16.3 net contraversive rotations in 30 (deprenyl) or 60 (BU224 and 2-BFI) min. In contrast, the reversible MAO-A inhibitor moclobemide (2.5–10 mg kg$^{-1}$) and the reversible MAO-B inhibitor lazabemide (2.5–10 mg kg$^{-1}$) failed to instigate significant rotational behaviour compared to vehicle.

**4** Coadministration of lazabemide (10 mg kg$^{-1}$), moclobemide (10 mg kg$^{-1}$) or 2-BFI (14 mg kg$^{-1}$) with L-DOPA (20 mg kg$^{-1}$) significantly increased either the duration or total number of contraversive rotations emitted over the testing period in comparison to L-DOPA alone.

**5** These data suggest that $I_2$-specific ligands have dual effects in the 6-OHDA-lesioned rat model of Parkinson's disease; a first effect associated with an increase in activity in the intact hemisphere, probably *via* an increase in striatal dopamine content, and a secondary action which, through the previously documented inhibition of MAO-A and/or MAO-B, increases the availability of dopamine produced by L-DOPA.

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**Keywords:** 2-BFI; deprenyl; imidazoline $I_2$ site; rat; *in vivo*; lazabemide; moclobemide; monoamine oxidase; Parkinson's disease

**Abbreviations:** 2-BFI, 2-(-2-benzofuranyl)-2-imidazoline; BU216, 3-[4,5-dihydroimidaz-2-yl]-quinoline hydrochloride; BU224, 2-[4,5-dihydroimidaz-2-yl]-quinoline; BU226, 2-[4,5-dihydroimidaz-2-yl]-isoquinoline hydrochloride; COMT, catechol-*O*-methyl transferase; L-DOPA, L-3,4-dihydroxyphenylamine; MAO, monoamine oxidase; 6-OHDA, 6-hydroxydopamine; PBS, phosphate-buffered saline; PD, Parkinson's disease; Ro41-1049, *N*-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxamide hydrochloride; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase

## Introduction

Imidazoline-binding sites (I sites) constitute a unique component of the binding profile of many imidazolines, guanidiniums and structurally related derivatives (Eglen *et al*., 1998). Such sites have been separated into at least three entities, imidazoline-1 ($I_1$), imidazoline-2 ($I_2$), imidazoline-3 ($I_3$), based on their respective preference for the $\alpha_2$-adrenoceptor ligands, clonidine, idazoxan and methoxy-idazoxan (Michel & Ernsberger, 1992; Chan *et al*., 1995). Recently, ligands have been developed with high selectivity for imidazoline $I_2$ sites, notably 2-BFI (2-(2-benzofuranyl)-2-imidazoline) and its quinoline and isoquinoline analogues, BU216 (3-[4,5-dihydroimidaz-2-yl]-quinoline hydrochloride), BU224 (2-[4,5-dihydroimidaz-2-yl]-quinoline) and BU226 (2-[4,5-dihydroimidaz-2-yl]-isoquinoline hydrochloride; Lione *et al*., 1998).

Autoradiographic studies with [$^3$H]idazoxan, [$^3$H]2-BFI and [$^3$H]BU224 indicate that $I_2$ sites exhibit a differential distribution in rat brain (MacInnes & Handley, 2001), with low levels of binding found throughout the basal ganglia motor loop (Lione *et al*., 1998; Robinson *et al*., 2002). The functional significance of the sites in the basal ganglia is unknown, since neither their molecular structure nor their second-messenger systems have been elucidated. However, there is extensive evidence that imidazoline $I_2$-binding sites exist on monoamine oxidase (MAO), at a location distinct from the catalytic site (Alemany *et al*., 1995; Raddatz *et al*., 1997; Remaury *et al*.,

*Author for correspondence; E-mail: susan.duty@kcl.ac.uk
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2000). *In vitro*, many imidazolines, including 2-BFI and BU224, reversibly inhibit MAO-A with a similar potency to that of the reversible MAO-A inhibitor moclobemide ($IC_{50}$: 2-BFI, $16.5 \pm 2.7\,\mu$M; BU224, $4.8 \pm 0.2\,\mu$M; moclobemide, $36 \pm 3.6\,\mu$M; Lalies *et al*., 1999). These imidazolines also similarly inhibit MAO-B, although with less potency than the selective reversible MAO-B inhibitor, lazabemide ($IC_{50}$: 2-BFI, $27.9 \pm 2.2\,\mu$M; BU224, $44.8 \pm 6.6\,\mu$M (Lalies *et al*., 1999); lazabemide, $0.03\,\mu$M (Da Prada *et al*., 1987)) and *in vivo* studies indicate that moclobemide, lazabemide and deprenyl show substitution for 2-BFI in an two-lever drug-discrimination paradigm (MacInnes & Handley, 2002). However, there appears to be little correlation between these agents' affinity for $I_2$ sites and their inhibition of MAO. Thus, despite their above-mentioned similar potencies against MAO-A, moclobemide displays negligible affinity for the $I_2$-binding site ($Ki > 100\,\mu$M) compared to the high affinity displayed by both 2-BFI (Ki 1.7 nM) and BU224 (Ki 2.1 nM) (Lione *et al*, 1998). This discrepancy suggests that 2-BFI and BU224 may bind to MAOs at sites distinct from that of established MAO inhibitors.

In Parkinson's disease (PD), degeneration of the nigrostriatal pathway results in reduced striatal dopamine levels and the single most effective treatment for this is L-DOPA (L-3,4-dihydroxyphenylamine). The therapeutic benefit of L-DOPA is ascribed to the central action of dopamine that is synthesised in the brain by decarboxylation of L-DOPA (Barbeau, 1981). Coadministration of a peripheral decarboxylase inhibitor helps to reduce peripheral side effects of L-DOPA but long-term treatment is still plagued by debilitating centrally mediated side effects, such as L-DOPA-induced dyskinesia (Nutt, 1990). For this reason, alternative treatments or refinements to existing ones are being investigated (Stocchi *et al*., 1997). Much attention has focused on the use of MAO-B inhibitors as adjuncts to L-DOPA treatment (Le Witt & Nyholm, 2004). Deprenyl, for example, has been shown to have therapeutic benefit when given alone in younger patients to defer the use of L-DOPA or as an adjunct to L-DOPA in the later stages to ameliorate L-DOPA-induced motor fluctuations (Parkinson Study Group 1994; 1996; Jankovic, 2000). Indeed a new generation of MAO inhibitors are currently being investigated both preclinically (e.g. Aubin *et al*., 2004) and in phase three clinical trials (rasagiline; Parkinson Study Group, 2004).

Previous radioligand-binding studies have indicated that $I_2$ sites, as defined by [$^3$H]2-BFI but not [$^3$H]idazoxan, are increased in the putamen of PD sufferers (Reynolds *et al*., 1996; Gargalidis-Moudanos *et al*., 1997). Given that many $I_2$-site ligands also inhibit MAO, this elevated density of $I_2$ sites may offer an additional treatment target. The aims of the present study were, therefore, to examine the ability of $I_2$-site ligands and specific MAO-A and MAO-B inhibitors to produce locomotor activity when administered alone or to enhance the effects of coadministered L-DOPA, in rats bearing a unilateral 6-OHDA lesion of the nigrostriatal tract.

## Methods

### *Production of 6-OHDA lesions of the nigrostriatal pathway*

A total of 16 male, Sprague–Dawley rats (Tucks, U.K.; 200–220 g) were housed in pairs in temperature- and humidity-controlled environment, on a 12 h light/dark cycle with free access to food and water. All procedures conformed to the U.K. Animals (Scientific Procedures) Act, 1986 and all efforts were made to minimise animals' suffering and the number of animals used. Unilateral 6-OHDA lesions of the nigrostriatal tract were produced as described previously (Chadha *et al*., 2000). At 30min prior to surgery, rats were injected with pargyline ($5\,mg\,kg^{-1}$; i.p., intraperitoneally) and desipramine ($25\,mg\,kg^{-1}$; i.p.) to elevate 6-OHDA availability and specificity for dopaminergic neurones. Under general anaesthesia (2.5% isoflurane in 95% $O_2$, 5% $CO_2$), animals were placed in a Kopf small animal stereotaxic frame, the mouthpiece set at 3.3 mm below the ear bars. A single injection of 6-OHDA ($12.5\,\mu$g in $2.5\,\mu$l sterile water containing 0.02% ascorbic acid, $1\,\mu l\,min^{-1}$) was made into the right median forebrain bundle (coordinates; 2.8 mm anterior, 2 mm lateral and 9 mm ventral to bregma) according to the rat brain atlas of Paxinos and Watson (1998). At 2 weeks after lesioning, animals were injected with amphetamine ($5\,mg\,kg^{-1}$) and placed in automated rotometers (Med. Associates) and rotations recorded for 60 min. Based on previous studies (Hefti *et al*., 1980; Murray *et al*., 2002), rats that exhibited $>50$ full ipsiversive rotations in the 10 min time-bin between 40 and 50 min postinjection were deemed suitably lesioned. These rats ($n = 14$) were randomly allocated into two groups of seven for inclusion in the subsequent studies. Administration of test compounds commenced 4 weeks after amphetamine challenge.

### *Administration of test compounds*

Animals were placed in automated rotometers (Med. Associates) and exposed to a 30 min adjustment period. The apparatus consisted of stainless steel bowls inside which each rat was placed in a jacket that was linked to an infrared sensor directly above the animal. The sensor detected the number of partial (45°) rotations ipsiversive and contraversive to the lesion and these data were recorded with ROTORAT software. When examining the effects of drug alone (deprenyl, 2-BFI, BU224, moclobemide or lazabemide), animals were administered the test compound and recording continued for up to 60 min. For the L-DOPA combination experiments, animals were administered either 2-BFI ($14\,mg\,kg^{-1}$), moclobemide ($10\,mg\,kg^{-1}$), lazabemide ($10\,mg\,kg^{-1}$) or vehicle combined with the peripheral decarboxylase inhibitor benserazide ($15\,mg\,kg^{-1}$), and then, 30 min later, L-DOPA ($10\,mg\,kg^{-1}$) was administered. Recording continued for a further 240 min. Drug treatments were distributed between groups as follows: Group 1; 2-BFI, moclobemide + L-DOPA, lazabemide + L-DOPA. Group 2; BU224, moclobemide, lazabemide, 2-BFI + L-DOPA, deprenyl. Based on our previous studies (MacInnes & Handley, 2002), single-drug experiments were conducted every other day, while L-DOPA combination studies were conducted on Mondays and Thursdays. Different doses, including vehicle control, were distributed across sessions and rats in pseudorandom order except for deprenyl, which, because it is an irreversible inhibitor, was given as the last dose to group 2. All drugs were dissolved in 0.9% physiological saline, except for moclobemide which was made up in deionised water, and administered i.p in a dose volume of $2\,ml\,kg^{-1}$. Doses of $I_2$-specific ligands and reversible MAO inhibitors were based on those that were effective in previous

drug discrimination studies (MacInnes & Handley, 2002) and that retained full solubility in saline. The dose of deprenyl (20 mg kg$^{-1}$) was chosen on the basis of previous preclinical studies (Heikkila *et al*., 1981; Prat *et al*., 2000) and was towards the higher end of the effective dose range to ensure maximum chance of obtaining an effect with only a single dose of this irreversible inhibitor. The dose of L-DOPA (10 mg kg$^{-1}$) was chosen on the basis of the previous L-DOPA potentiation studies of (Heeringa *et al*., 1997).

### *Verification of lesion by tyrosine hydroxylase (TH) immunohistochemistry*

At 24 h after administration of the final test drug, rats were deeply anaesthetised (pentobarbitone 60 mg kg$^{-1}$, i.p.) and perfused *via* the left ventricle with 100 ml ice-cold phosphate buffered saline (PBS) (0.1 M; pH 7.4) followed by 100 ml ice-cold PBS containing 4% paraformaldehyde. Brains were immediately removed and postfixed for a further 48 h in 0.1 M PBS containing 4% paraformaldehyde at 4°C. Brains were cryoprotected in 30% sucrose for up to 96 h or until brains sank and then 30 $\mu$m coronal sections of the substania nigra were cut using a freezing microtome. Sections were stored in 0.1 M PBS containing 0.05% sodium azide until assay. Sections were labelled with TH -specific antibody according to the protocol of Iravani *et al*. (2002). The extent of nigral lesion was derived from comparison of the mean number of TH immunoreactive cell bodies detected under light microscopy between the lesion and nonlesion hemispheres (3–4 sections per rat).

### *Statistical analysis*

For single-injections studies, rotational behaviour was compared between doses of a given drug or vehicle using a repeated measures one-way analysis of variance with Student–Newman–Keuls *post hoc* test (GraphPAD Prism version 3) or, for deprenyl alone, using paired *t*-tests after confirming that there were no deviations from Gaussian distribution. For L-DOPA combination studies, the total number and duration of rotations produced by L-DOPA + drug were compared to those of L-DOPA + vehicle using repeated measures two-way analysis of variance with a Dunnett's *post hoc* test. Unless otherwise indicated, data represent mean ± standard error of the mean (s.e.m.)

### *Drugs*

BU224 (2-[4,5-dihydroimidaz-2-yl]-quinoline hydrochloride) was donated by Alan Hudson, Bristol University, U.K; moclobemide (*p*-chloro-*N*-(2-morpholinoethyl) benzamide) and lazabemide (*N*-(2-aminoethyl)-5-chloro-2-pyridinecarboxamide hydrochloride) were donated by Hoffman La Roche, Switzerland. 2-BFI (2-(-2-benzofuranyl)-2-imidazoline) was purchased from Tocris, U.K. Deprenyl, 6-OHDA (6-hydroxydopamine, pargyline, desipramine, L-DOPA (L-3,4-dihydroxyphenylamine), benserazide, amphetamine and all other reagents were purchased from Sigma, U.K. All drugs were dosed as HCl salts, except for 6-OHDA which was dosed as the HBr salt.

## Results

### *Lesion verification*

All rats that had undergone surgery to produce a 6-OHDA lesion were screened prior to inclusion in the following studies by administration of 5 mg kg$^{-1}$ amphetamine. Out of the 16 rats screened, 14 exhibited robust ipsiversive rotational behaviour (4111 ± 729 partial rotations/86 ± 12 full rotations in 10 min; $n = 14$) in response to amphetamine challenge. After experimentation was completed, assessment of TH immunoreactivity confirmed that these animals had over 93% loss of dopamine cell bodies in the SNc (substantia nigra pars compacta) between the lesion (6.7 ± 0.9, cells per SNc; $n = 14$,) and nonlesion (104.6 ± 5.9, cells per SNc; $n = 14$) hemispheres.

### *Single-injection studies*

As shown in Figure 1, significant increases in ipsiversive rotational behaviour compared to vehicle were produced following the administration of 2-BFI (1-way ANOVA; $F(3,18) = 4.79$, $P < 0.05$) and BU224 (1-way ANOVA; $F(3,18) = 4.07$, $P < 0.05$). The Student–Newman–Keuls *post hoc* analysis revealed that the responses to 7 and 14 mg kg$^{-1}$ 2-BFI and 14 mg kg$^{-1}$ BU224 reached significance with maximum net partial ipsiversive rotations of 92.5 ± 16.3 in 60 min and 131.7 ± 37.2 in 60 min being achieved, respectively. Deprenyl (20 mg kg$^{-1}$) also produced a significant increase in net partial ipsiversive rotations compared to vehicle ($T(6) = 3.51$; $P < 0.05$), achieving 520.7 ± 120.5 rotations in 30 min. In contrast, the reversible MAO-A inhibitor moclobemide ($F(3,18) = 0.30$, $P = 0.82$) and the reversible MAO-B inhibitor lazabemide ($F(3,18) = 0.49$, $P = 0.68$) failed to elicit significant increases in ipsiversive rotations in comparison to vehicle.

### *L-DOPA combination studies*

In comparison to L-DOPA (10 mg kg$^{-1}$) alone, the $I_2$-specific ligand 2-BFI (14 mg kg$^{-1}$) + L-DOPA (10 mg kg$^{-1}$) yielded a significant main effect for treatment ($F(1,180) = 34.78$, $P < 0.0001$) and time ($F(29,180) = 6.87$, $P < 0.0001$), and Dunnett's *post hoc* test indicated that 2-BFI significantly increased the total number of partial contraversive rotations (Figure 2a) and the duration of this rotational behaviour (Figure 2c). Consistent with the single-drug studies reported above, the administration of 2-BFI significantly increased the number of ipsiversive rotations that occurred in the two 10-min time bins directly after its administration, as reflected by the negative dip in net contraversive rotations (Figure 2c).

Administration of L-DOPA (10 mg kg$^{-1}$) either alone or in combination with 10 mg kg$^{-1}$ moclobemide or lazabemide gave rise to a significant main effect for treatment ($F(2,360) = 27.02$, $P < 0.0001$) and time ($F(29,360) = 6.56$, $P < 0.0001$). Dunnett's *post hoc* test indicated that the lazabemide + L-DOPA combination produced significantly more partial contraversive rotations over the 240 min recording period than L-DOPA alone. In contrast, the potentiating effect of moclobemide over this whole period just failed to reach significance (Figure 2b). However, both moclobemide and lazabemide significantly

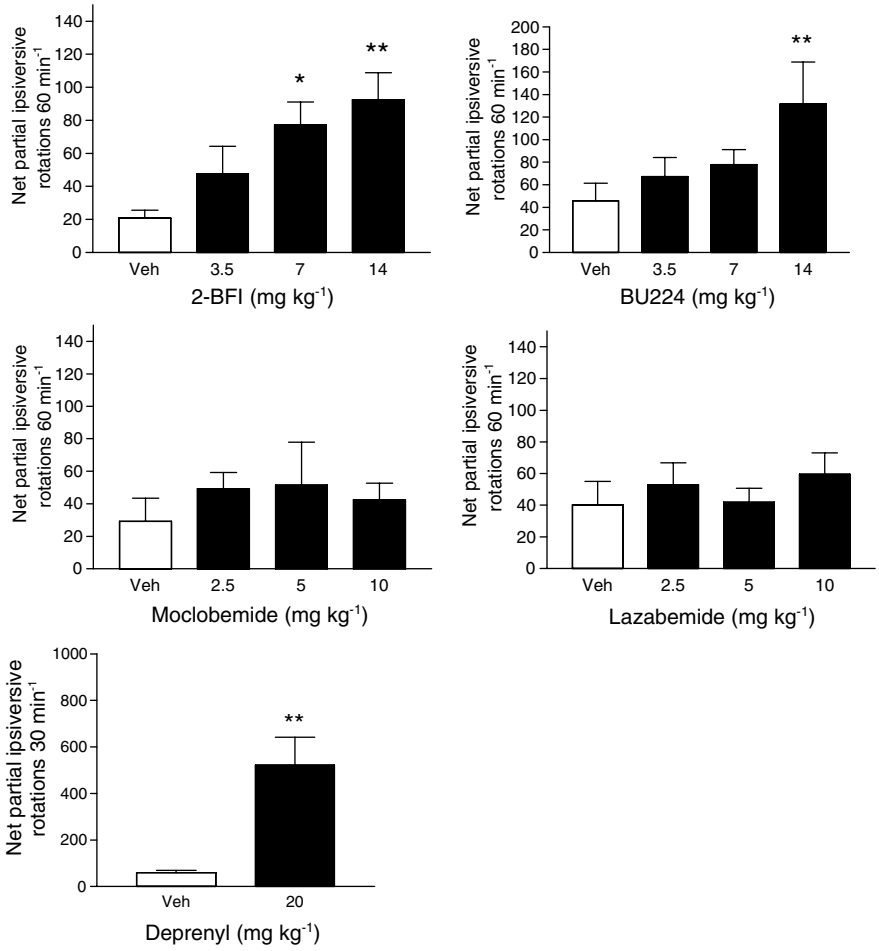


**Figure 1** Ability of i.p. administration of $I_2$-site ligands (2-BFI and BU224) and MAO inhibitors (moclobemide, lazabemide and deprenyl) to elicit ipsiversive rotations in rats bearing a unilateral 6-OHDA lesion. Rotational behaviour was measured for 30 or 60 min post drug or vehicle administration. Data are mean ± s.e.m. ($n = 7$). $^*P < 0.05$ and $^{**}P < 0.01$ indicate a statistically significant difference from vehicle using either the Student–Newman–Keuls test after a significant one-way ANOVA or a paired *t*-test (deprenyl alone).

increased the duration of L-DOPA-induced rotational behaviour (Figure 2d) compared to that seen with L-DOPA alone.

## Discussion

The data presented here show, for the first time, that administration of the $I_2$-specific ligands, 2-BFI and BU224, produce ipsiversive rotational behaviour in rats bearing a full 6-OHDA lesion of the nigrostriatal tract. The full extent of the 6-OHDA lesion was evidenced in two ways: firstly, by the production of marked ipsiversive rotations with 5 mg kg$^{-1}$ amphetamine, which, in animals bearing a sham lesion, would produce no ipsiversive rotations (Murray *et al*., 2002) and secondly, by the loss of >93% TH-positive cells in the SNc of the lesioned hemisphere in contrast to a sham lesion where no significant loss of TH-positive cell numbers is seen from a similar baseline of ~100 cells per SNc (O'Neill *et al*., 2004). The degree of rotations produced by both $I_2$-specific ligands, although small, was significantly elevated compared to that produced by vehicle alone. Additionally, over the dose range studied, a plateau response and hence true maximum effect was not reached, thus it is possible that much larger rotational responses may be produced with increased doses of 2-BFI and BU224. That vehicle alone elicits a low level of spontaneous ipsiversive rotational behaviour in these animals is consistent with other studies of this type and is thought to reflect basal levels of dopamine release in the intact hemisphere induced by the injection procedure *per se* (Chopin *et al*., 1999).

Triggering release of dopamine from nigrostriatal neurones in the intact hemisphere is a well-established and robust means of producing ipsiversive rotations in unilateral lesioned rats (e.g. Ungerstedt, 1971; Pycock, 1980). This phenomenon is further evidenced in the present study by the above-mentioned marked degree of ipsiversive rotations produced by the dopamine-releasing agent amphetamine. It follows that one possible explanation for the ipsiversive rotational behaviour seen here with 2-BFI and BU224 is that these $I_2$-site ligands may also act to release dopamine in the striatum of the intact hemisphere. That $I_2$-site ligands may act as 'dopamine releasers' has been suggested previously (Sastre-Coll *et al*., 2001) and is backed up by the *in vivo* microdialysis studies of Hudson *et al*. (1999), which showed that acute administration of similar doses of 2-BFI and BU224 as used here (20 mg kg$^{-1}$) increased extrasynaptic levels of dopamine in the striatum by 0.5- and 2.5-fold above baseline, respectively. That this

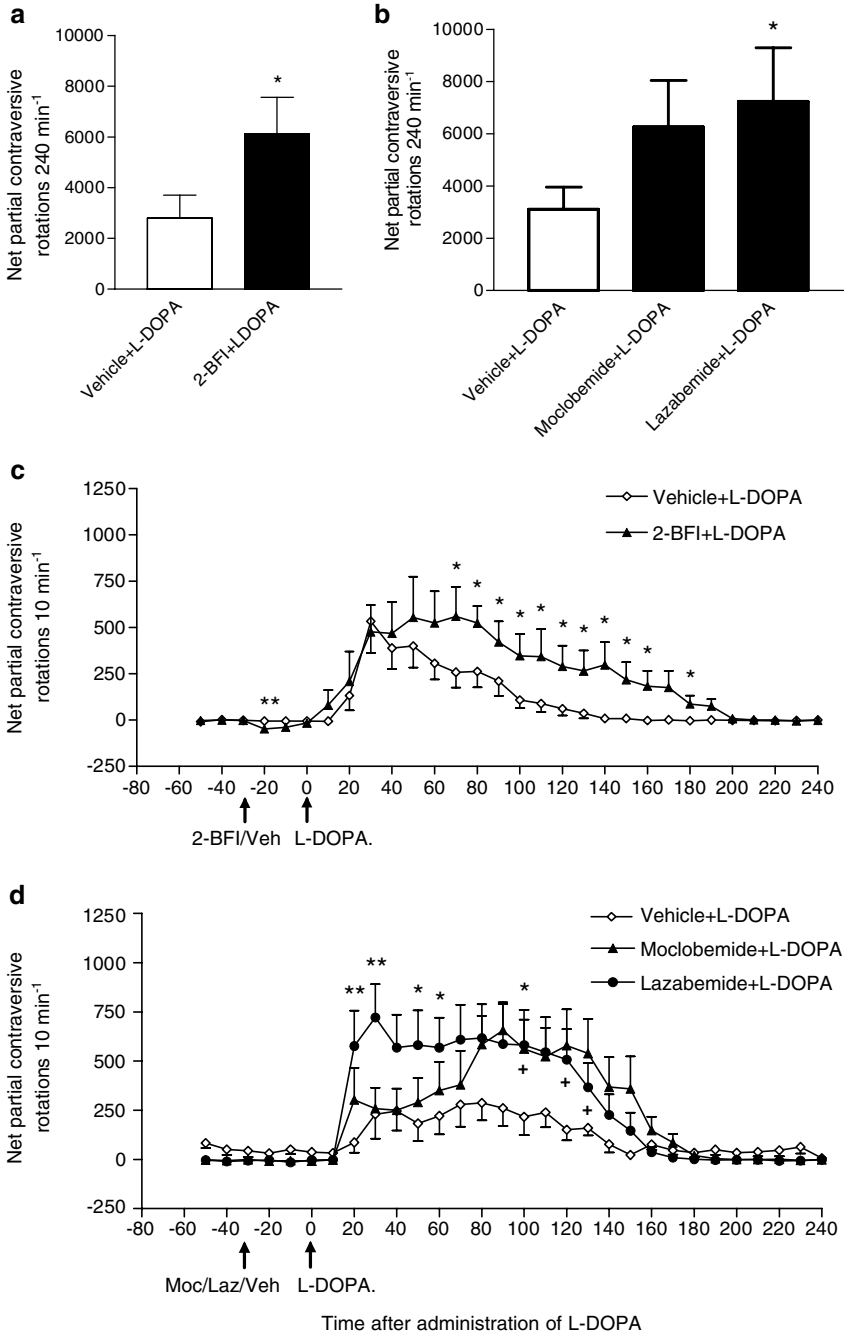


**Figure 2** Ability of the $I_2$-site ligand 2-BFI (14 mg kg$^{-1}$ i.p.) or the MAO inhibitors, moclobemide (10 mg kg$^{-1}$ i.p.) and lazabemide (10 mg kg$^{-1}$ i.p.) to potentiate L-DOPA (10 mg kg$^{-1}$ i.p.)-induced contraversive rotations in rats bearing a unilateral 6-OHDA lesion. (a, b) Total number of rotations over 240 min are shown. *$P<0.05$ indicates a statistically significant difference between drug + L-DOPA *versus* vehicle + L-DOPA using either a paired *t*-test (a) or Dunnett's test after a significant two-way ANOVA (b). (c, d) Animals were administered test drugs in conjunction with benserazide (15 mg kg$^{-1}$) at time *T*-30 min, with 10 mg kg$^{-1}$ L-DOPA being given 30 min later at time *T*0. In (c), *$P<0.05$ indicates a statistically significant difference between 2-BFI + L-DOPA *versus* vehicle + L-DOPA (paired *t*-test; after a significant 2-way ANOVA). In (d), *$P<0.05$, and **$P<0.01$ indicate a statistically significant difference between lazabemide + L-DOPA *versus* vehicle + L-DOPA; $^{+}P<0.05$ indicates a statistically significant difference between moclobemide + L-DOPA *versus* vehicle + L-DOPA (Dunnett's test after a significant two-way ANOVA). Data are mean ± s.e.m. ($n=7$). Abbreviations: Laz, lazabemide. Moc, moclobemide. Veh, vehicle.

increase in striatal dopamine levels is small compared to the 10-fold increase above baseline that would be achieved by 5 mg kg$^{-1}$ amphetamine (Lamensdorf *et al*., 1999) is consistent with the level of rotations achieved with 2-BFI and BU224 being small compared to that produced by amphetamine. Moreover, that BU224 (20 mg kg$^{-1}$) produced a greater elevation of extracellular striatal dopamine than an identical dose of 2-BFI (Hudson *et al*., 1999) is also consistent with the presently observed number of ipsiversive rotations (~131) produced by a similar dose of BU224 (14 mg mg kg$^{-1}$) being

greater than that produced by an identical dose of 2-BFI (~92). Taken together, these data support a correlation between these two events, elevation of extracellular striatal dopamine and ipsiversive rotational behaviour.

An increase in extracellular striatal dopamine levels could, of course, reflect many things in addition to increasing dopamine release, such as reduced reuptake of dopamine or reduced metabolism *via* catechol-*O*-methyl transferase (COMT) or MAO, especially considering that this previously documented rise in striatal dopamine levels was accompanied by a concomitant decrease in the levels of dopamine metabolites, homonovanillic acid and 3,4-dihydroxyphenylacetic acid (Hudson *et al*., 1999). Since no study has yet shown that the $I_2$-specific ligands can either bind to or inhibit COMT, a contribution from this source is unlikely, although remains possible. In contrast, it is well established, as outlined earlier, that both 2-BFI and BU224 can inhibit MAO-A and MAO-B and the profile of increased dopamine levels and reduced dopamine turnover is indeed similar to that previously reported for inhibitors of MAO (Kato *et al*., 1986; Burkward *et al*., 1989; Butcher *et al*., 1990). However, since neither of the reversible MAO-A or MAO-B inhibitors used in the present study (moclobemide or lazabemide) instigated ipsiversive rotational behaviour in the 6-OHDA lesioned rat when given alone, such enzyme inhibition seems unlikely to underpin the rotational behaviour seen with 2-BFI or BU224 given alone. In further support of this, the MAO inhibitor deprenyl seen here and at this same dose in previous studies to elicit ipsiversive rotations when administered alone in 6-OHDA-lesioned rats (Heikkila *et al*., 1981), is believed to do so through its ability to enhance dopamine release and/or inhibit dopamine reuptake, as demonstrated *in vitro* in striatal slices (e.g. Heikkila *et al*., 1981; Fang & Yu, 1994; Neusch *et al*., 1997), rather then *via* MAO inhibition (Finberg & Youdim, 1994). Since the ability of $I_2$-site ligands to interfere with dopamine uptake mechanisms has not yet been investigated, such an action cannot be discounted as potentially contributing to the proposed elevation in striatal extracellular dopamine levels.

Ipsiversive rotations may also be elicited *via* blockade of presynaptic $\alpha_2$-adrenoceptors on nigrostriatal terminals, leading to facilitation of dopaminergic transmission in the intact hemisphere. Such a mechanism underlies the rotational response to the $\alpha_2$-adrenoceptor antagonist efaroxan, which itself also contains the imidazoline moiety (Chopin *et al*., 1999). However, such an action is again unlikely to underlie the ipsiversive rotational response of the $I_2$-site ligands since, at least for 2-BFI, the doses tested here have negligible affinity for $\alpha_2$-adrenoceptors *in vitro* (Nutt *et al*., 1995) and fail to inhibit $\alpha_2$-adrenoceptor-mediated responses *in vivo* (Jordan *et al*., 1996). Thus, while enhanced extracellular striatal dopamine levels remain the most likely mediator of the ipsiversive rotational response of $I_2$-site ligands in the 6-OHDA lesioned rat, the exact cellular mechanisms underlying this enhancement remain to be fully established.

The present study also demonstrated for the first time that coadministration of the specific $I_2$-site ligand 2-BFI with L-DOPA lead to a potentiation of L-DOPA-induced contraversive rotational behaviour in rats bearing a full 6-OHDA lesion of the nigrostriatal tract. L-DOPA instigates contraversive rotational behaviour in the unilateral 6-OHDA-lesioned rat *via* a well-established mechanism. Thus, while peripheral administration of L-DOPA increases dopamine on both sides of the brain, its action at the supersensitive dopamine receptors within the denervated striatum leads to an exaggerated response in the lesioned hemisphere that culminates in contraversive rotational behaviour. Coadministration of an MAO inhibitor such as deprenyl potentiates the actions of L-DOPA by preventing dopamine breakdown (Heikkila *et al*., 1981; Prat *et al*., 2000). The present study confirmed these findings with the reversible MAO-A inhibitor, moclobemide and the reversible MAO-B inhibitor, lazabemide, both of which significantly increased the duration of L-DOPA-induced contraversive rotations, while lazabemide also significantly increased the total number of rotations produced by L-DOPA. While previous studies have shown that another reversible MAO-A inhibitor, Ro41-1049 (*N*-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxamide hydrochloride), also increases the duration of L-DOPA-induced contraversive rotations (Heeringa *et al*., 1997), these same authors failed to demonstrate a significant effect of lazabemide to alter either the duration or number of L-DOPA-induced rotations (Heeringa *et al*., 1997). This discrepancy in lazabemide's efficacy may lie with the timing of administration of the peripheral aromatic amino-acid decarboxylase inhibitor, benserazide. In the present study, benserazide was administered 30 minutes before L-DOPA, thereby maximising the quantity of L-DOPA that reaches the brain. In contrast, Heeringa *et al*. (1997) administered benserazide at the same time as L-DOPA, potentially resulting in more L-DOPA being converted to dopamine in the periphery, thereby providing less chance for L-DOPA conversion to dopamine in the brain.

As previously described, it is well established that $I_2$-binding sites exist on MAO (Alemany *et al*., 1995; Raddatz *et al*., 1997; Remaury *et al*., 2000) and that $I_2$-site ligands including 2-BFI reversibly inhibit MAO-A and MAO-B activity *in vitro* (Ozaita *et al*., 1997; Lalies *et al*., 1999). Therefore, the ability of 2-BFI to inhibit MAO most likely underlies its ability to potentiate both the duration and number of L-DOPA-induced contraversive rotations in the 6-OHDA-lesioned rat. Although it will be important to replicate these findings with other $I_2$ ligands such as BU224, to strengthen this supposition, given the similar potencies of BU224 and 2-BFI to inhibit MAO and their similar profiles of action when given alone to 6-OHDA lesioned rats, it seems reasonable, at this stage, to predict that BU224 might also potentiate the actions of L-DOPA in these animals.

## Conclusions

The present study confirms the findings of others that administration of MAO-A and MAO-B inhibitors potentiates L-DOPA-induced contraversive rotational behaviour in the 6-OHDA-lesioned rat and extends these finding to include the $I_2$-specific ligand, 2-BFI. In addition, the study also demonstrates that the $I_2$-site-specific ligands, 2-BFI and BU224, are able to induce ipsiversive rotations when administered alone in the 6-OHDA-lesioned rat. These data suggest that $I_2$-specific ligands may have dual effects in the 6-OHDA-lesioned rat model of PD; an immediate effect associated with an increase in activity in the intact hemisphere, probably *via* an increase in striatal dopamine levels, and a secondary action which, through the

previously documented inhibition of MAO-A and/or MAO-B, increases the availability of dopamine produced by L-DOPA. This pharmacological profile suggests that $I_2$-specific ligands may be worthy of further investigation as alternative adjuncts to L-DOPA in the treatment of PD.

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