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L-Dopa esters as potential prodrugs: behavioural activity in experimental models of Parkinson's disease‡

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Intraperitoneal administration of the 2-tetrahydropyranylmethyl, phenoxyethyl, ethyl, 2-hydroxypropyl and methyl ester prodrugs of L-dopa produced locomotor activity in reserpine-pretreated mice with equal intensity and duration to that observed following administration of L-dopa itself. Administration of the 2-(1-methoxy)propyl ester produced a more prolonged effect while the *p*-methoxyphenylethyl, n-propyl, phenylethyl, *m*-trifluoromethylbenzyl, cyclohexyl, *p*-chlorophenylethyl and benzyl ester prodrugs were less active than L-dopa itself. On oral administration, the ethyl and methyl ester prodrugs were more effective than L-dopa in reversing reserpine-induced akinesia in mice. The 2-tetrahydropyranylmethyl, 2-(1-methoxy)propyl, 2-hydroxypropyl, n-propyl, benzyl and phenoxyethyl ester prodrugs produced effects comparable with those of L-dopa. In contrast, the cyclohexyl, *m*-trifluoromethylbenzyl, phenylethyl, *p*-chlorophenylethyl and *p*-methoxyphenylethyl ester prodrugs were less effective than L-dopa on oral administration. Intraperitoneal administration of L-dopa and the ester prodrugs of L-dopa to rats with a prior 6-hydroxydopamine (6-OHDA) lesion of the medial forebrain bundle (MFB) produced contraversive circling responses. Rotation observed following administration of the n-propyl, 2-tetrahydropyranylmethyl, methyl and ethyl ester prodrugs was more intense than that observed following administration of L-dopa itself. Rotation produced by the administration of L-dopa and the cyclohexyl, 2-(1-methoxy)propyl, phenylethyl, *p*-chlorophenylethyl, *p*-methoxyphenylethyl, benzyl, 2-hydroxypropyl, phenoxyethyl and *m*-trifluoromethylbenzyl ester prodrugs was identical. Ester prodrugs of L-dopa may be as effective as L-dopa itself in producing motor activity but overall none of the compounds tested was markedly more potent or of longer duration than L-dopa itself.

Parkinson's disease is characterized by a general loss of dopamine neurons in brain (see Hornykiewicz 1982). Dopamine deficiency appears to be responsible for the motor deficits of the disorder since administration of the precursor L-3,4-dihydroxyphenylalanine (L-dopa) or synthetic dopamine agonist drugs, such as bromocriptine and pergolide, restores function. At present L-dopa remains the mainstay of treatment for Parkinson's disease.

However, during chronic treatment with L-dopa, a variety of problems may emerge. Patients experience a decrease in the duration of drug effect ('wearing-off' phenomenon) and rapid fluctuations in response ('on–off' phenomenon). These phenomena are related, at least in part, to fluctuations in L-dopa plasma levels; when plasma L-dopa levels are low or falling, patients lose benefit from the drug. Recent studies of continuous infusions of L-dopa shows that stable plasma L-dopa levels can maintain mobility in parkinsonian patients (Shoulson et al 1975; Quinn et al 1982, 1984; Hardie et al 1984).

L-Dopa itself has poor bioavailability and a relatively short half-life in-vivo. It has not been easy to produce a sustained release preparation of L-dopa capable of more effectively maintaining adequate plasma levels. For this reason there has been interest in developing a prodrug derivative of L-dopa which would be well-absorbed and slowly converted to L-dopa in-vivo. As early as 1965, Hanson & Utley studied the methyl ester of L-dopa as a possible prodrug, but this and other similar attempts have not led to a more useful dopamimetic agent (Lai & Mason 1973; Brossi et al 1974; Felix et al 1974; Bodor et al 1977).

We have examined the behavioural activity of a series of potential prodrugs of L-dopa in two experimental animal models of Parkinson's disease. Thus we have estimated the ability of prodrugs of L-dopa to reverse the akinesia induced in mice by

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‡ Part III of the series on L-dopa esters as potential prodrugs.

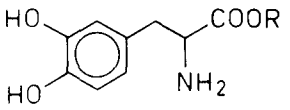
reserpine, an effect first observed for L-dopa (Carlsson et al 1957), and to induce circling behaviour in rats with a 6-OHDA lesion of the medial forebrain bundle (MFB) (Ungerstedt 1971). A preliminary report on the pharmacological effects of the methyl ester of L-dopa has already been published (Cooper et al 1984).

MATERIALS AND METHODS

Drugs

L-Dopa (Roche Products Ltd); ester prodrugs of L-dopa (1) as hydrochloride salts (methyl (2), ethyl (3), n-propyl (4), 2-hydroxypropyl (5), 2-(1-methoxy)propyl (6), cyclohexyl (7), 2-tetrahydropyranylmethyl (8), benzyl (9), *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) *p*-methoxyphenylethyl (13), and phenoxyethyl (14)) (Marrel et al 1985a, b; Table 1); reserpine

Table 1. Chemical structures of L-dopa and ester prodrugs of L-dopa.



	-R
1. L-Dopa	-H
2. Methyl	$-CH_3$
3. Ethyl	$-CH_2CH_3$
4. n-Propyl	$-CH_2CH_2CH_3$
5. 2-Hydroxypropyl	$-CH_2CHOHCH_3$
6. 2-(1-Methoxy)propyl	$-CHCH_3CH_2OCH_3$
7. Cyclohexyl	$-\overline{CH\text{-}(CH_2)_4\text{-}CH_2}$
8. 2-Tetrahydropyranylmethyl	$-CH_2\text{-}\overline{CH\text{-}(CH_2)_4\text{-}O}$
9. Benzyl	$-CH_2\text{-}C_6H_5$
10. *m*-Trifluoromethylbenzyl	$-CH_2\text{-}C_6H_4\text{-}m\text{-}CF_3$
11. Phenylethyl	$-CH_2CH_2\text{-}C_6H_5$
12. *p*-Chlorophenylethyl	$-CH_2CH_2\text{-}C_6H_4\text{-}p\text{-}Cl$
13. *p*-Methoxyphenylethyl	$-CH_2CH_2\text{-}C_6H_4\text{-}p\text{-}OCH_3$
14. Phenoxyethyl	$-CH_2CH_2\text{-}O\text{-}C_6H_5$

(Halewood Chemicals Ltd); carbidopa (α-methyldopahydrazine; Merck, Sharp & Dohme Ltd); chloral hydrate (British Drug Houses); 6-hydroxydopamine hydrobromide (Sigma Chemical Co. Ltd); (+)-amphetamine sulphate (Smith, Kline & French Ltd); apomorphine hydrochloride (Evans Medical Ltd).

Reversal of reserpine-induced akinesia in mice

Reserpinized male mice (Tuck No. 1, 20–25 g; 5 mg kg^{-1} i.p., 18–24 h before) received carbidopa 25 mg kg^{-1} i.p., suspended in 1% w/v methylcellulose, and were placed in groups of 3 in activity cages (LKB Farad Animex Activity Meters or Motron Products Motility Meters). After a 60 min acclimatization period L-dopa (200 mg kg^{-1} equivalent to 1 mmol kg^{-1} i.p.; dissolved in 5 parts 0·9% w/v NaCl containing 0·2 M HCl and buffered with 2 parts 7% w/v $NaHCO_3$), or an equimolar dose of prodrug (dissolved in glass-distilled water), or vehicle was administered orally or intraperitoneally. Drugs were administered in a volume of 0·15 mL per 10 g body weight. The dose of L-dopa was chosen from pilot experiments as one which consistently reversed reserpine-induced akinesia. In a typical experiment L-dopa (1 mmol kg^{-1} i.p.) produced activity counts of 8398 ± 2205 in a 4 h period compared with reserpinized control animals who produced 424 ± 152 counts in 4 h ($P < 0.001$).

Following drug administration, activity was recorded over the subsequent 4 h as the mean (±1 s.e.m.) number of counts occurring in each 10 min period and the mean (±1 s.e.m.) total activity for the 4 h period for six batches of three mice.

In all experiments, a direct comparison was made on the same occasion between L-dopa and a prodrug to allow for any possible inter-experiment variation. In each individual experiment the L-dopa- and prodrug-treated animals were rotated through the various activity meters used to avoid differences in apparatus sensitivity.

Statistical evaluation was carried out by two-tailed two-way analysis of variance with replicates (ANOVA) and two-tailed unpaired Student's *t*-test for the comparison of mean (±1 s.e.m.) peak activities.

6-OHDA lesions of the medial forebrain bundle in rats

Female Wistar rats (170–190 g; Charles River UK Ltd) were anaesthetized with chloral hydrate (300 mg kg^{-1} i.p.) and positioned in a Kopf sterotactic frame. 6-OHDA (8 μg in 3 μL ice-cold sterile 0·9% w/v NaCl (saline) containing 2 μg ascorbic acid) or vehicle was injected into the brain through a Hamilton syringe (5 μL capacity) with Luer type needle (o.d. 0·33 mm, i.d. 0·18 mm) at a rate of 1 μL min^{-1}. The needle was left in position for 1 min following injection. The lesion site was the left ascending medial forebrain bundle (MFB) at the level of the lateral hypothalamus using the coordinates A 4·6, L 1·9, V −1·9 (De Groot 1959).

Biochemical and histological verification of MFB lesions

Biochemical assessment of the extent of MFB lesions was made by measurement of striatal dopamine concentration at least 3 weeks following surgery.

Rats were killed by cervical dislocation and decapitation. Brains were rapidly removed onto ice and the corpus striata were dissected out, frozen on an ice block and weighed. Tissue samples were prepared and dopamine separated from its acidic metabolites according to the method of Early & Leonard (1978). Determination of dopamine content was by the fluorimetric method of Laverty & Sharman (1965).

In rats with 6-OHDA-induced lesions of the MFB the dopamine content of the ipsilateral striatum was 741 ± 139 ng g^{-1} compared with a value for the contralateral striatum of 11 069 ± 609 ng g^{-1} ($P < 0.001$; Student's t-test). The dopamine content of the contralateral striatum was not different from that of control (sham-lesioned) animals (control left striatum 9978 ± 353 ng g^{-1}, $P > 0.05$; control right striatum 11 661 ± 1039 ng g^{-1}, $P > 0.05$).

Histological examination of the substantia nigra demonstrated the disappearance of cells in the pars compacta ipsilateral to the 6-OHDA lesion of the MFB and the presence of glial infiltration.

Circling behaviour in rats

Circling behaviour was examined 2 weeks following surgery. Circling rates (turns min^{-1}) were counted over a 1 min period, in an open field environment, 30 min following administration of (+)-amphetamine sulphate (3 mg kg^{-1} i.p. in distilled water) or 15 min following administration of apomorphine hydrochloride (0·5 mg kg^{-1} s.c. in 0·1% w/v sodium metabisulphite). Animals that turned at less than 8 turns min^{-1} were excluded. The animals used in this study exhibited contraversive circling behaviour of 16·5 ± 1·0 turns min^{-1} following apomorphine (0·5 mg kg^{-1} s.c.) administration, and ipsiversive circling behaviour of 16·4 ± 1·9 turns min^{-1} following amphetamine (3 mg kg^{-1} i.p.) administration.

In subsequent experiments the effect of administration of L-dopa or its prodrugs on circling behaviour was measured at least 3 weeks following surgery. Rats received carbidopa (25 mg kg^{-1} i.p., suspended in methylcellulose) and were placed in individual Perspex cages (20 × 18 × 18 cm). After 1 h, L-dopa (50 mg kg^{-1} i.p., equivalent to 0·25 mmol kg^{-1}, dissolved as above) or a prodrug (dissolved in glass-distilled water) in an equimolar dose to L-dopa or vehicle was administered. This dose of L-dopa was chosen because it consistently produced contralateral circling behaviour in rats with a 6-OHDA-induced lesion of the MFB in pilot experiments. Circling behaviour was measured for a 1 min period every 15 min for the duration of drug activity. Data was expressed as the mean (±s.e.m.) circling rate (turns min^{-1}) for at least 6 animals. Total activity was also expressed as the total number of turns measured, at all time points, for the duration of drug activity. Every rat was used on only three occasions receiving L-dopa and two prodrugs examined at intervals of 1 week.

Statistical analysis was carried out using a two-tailed two-way analysis of variance with replicates and by an unpaired two-tailed Student's t-test for comparison of mean (±s.e.m.) peak activities.

RESULTS

Reversal of reserpine-induced akinesia in mice following intraperitoneal administration of L-dopa or prodrugs

Naive mice (reserpine vehicle, 18–24 h before) exhibited intense locomotor activity during the first hour after introduction into the activity meters followed by a gradual decline over the remainder of the 4 h test period. In contrast, mice that received reserpine (5 mg kg^{-1} i.p., 18–24 h before) showed only a low level of activity throughout the time-course of the experiment. These mice appeared akinetic with varying degrees of tremor, piloerection and ptosis. Following treatment with vehicle or reserpine total activities for the 4 h period were 8585 ± 582 and 990 ± 330 counts, respectively ($P < 0.001$).

Following intraperitoneal administration of L-dopa (1 mmol kg^{-1}) plus carbidopa (25 mg kg^{-1} i.p.; 1 h before) to reserpinized (5 mg kg^{-1} i.p.; 18–24 h before) mice a consistent reversal of akinesia was observed. The mice exhibited increased locomotor activity 10 min following drug administration. This effect reached peak levels after approximately 1 h and lasted for up to 4 h. Over the 4 h test period the activity of mice receiving L-dopa plus carbidopa was 8398 ± 2205 compared with 8585 ± 582 counts for control mice receiving reserpine vehicle examined in parallel ($P > 0.05$).

The intraperitoneal administration of the ester prodrugs in equimolar doses to L-dopa plus carbidopa (25 mg kg^{-1} i.p., 1 h before) produced variable effects on reserpine-induced akinesia in the mice (examples are shown in Fig. 1a–d).

The 2-tetrahydropyranylmethyl (8), phenoxyethyl (14), 2-(1-methoxy)propyl (6), ethyl (3), 2-hydroxypropyl (5) and methyl (2) ester prodrugs (1 mmol kg^{-1} in each case) were equiactive compared with L-dopa (1) (Table 2). The duration and intensity of locomotor activity following administration of L-dopa (1) and the 2-tetrahydropyranylmethyl (8), phenoxyethyl (14), ethyl (3), 2-hydroxypropyl (5) and methyl (2) prodrug esters were virtually identical

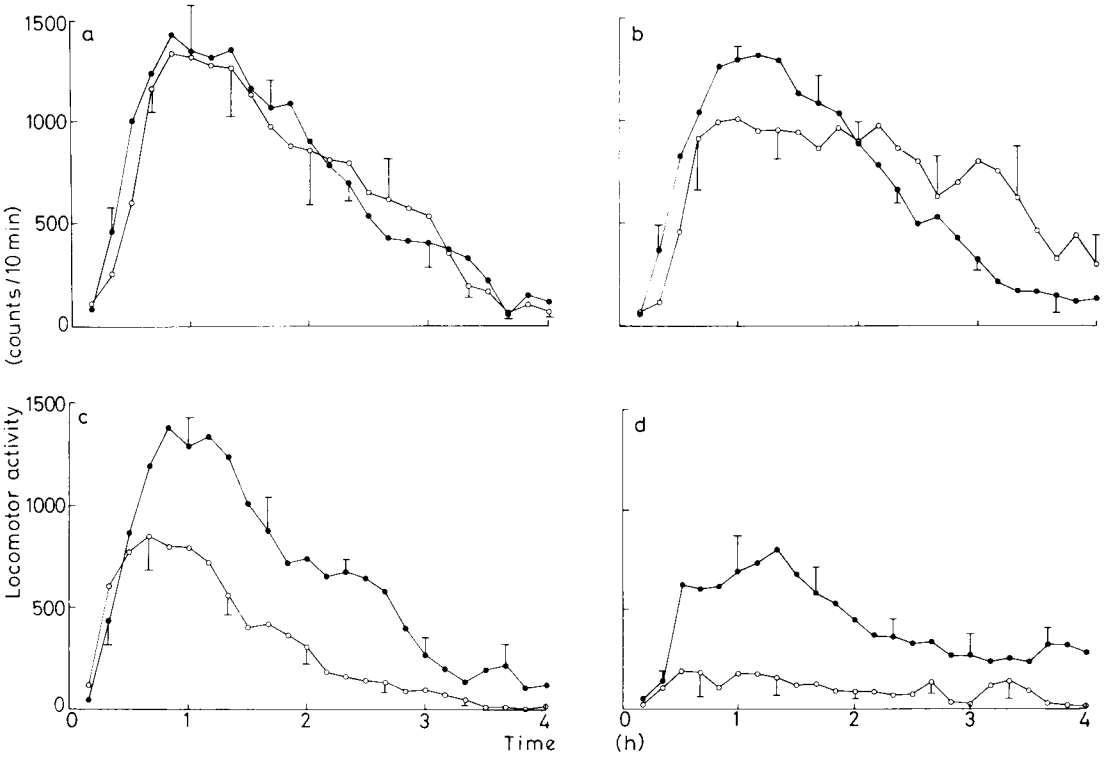


FIG. 1. Reversal of reserpine-induced akinesia in mice following intraperitoneal administration of equimolar doses of L-dopa (1 mmol kg^{-1}, —●—) and prodrug ester (1 mmol kg^{-1}, —○—) (a) methyl (2), (b) 2-(1-methoxy)propyl (6), (c) *m*-trifluoromethylbenzyl (10), and (d) benzyl (9) esters. All animals received carbidopa (25 mg kg^{-1}, i.p., 1 h before).

Table 2. Reversal of reserpine-induced akinesia in mice by intraperitoneal administration of L-dopa (1 mmol kg^{-1}) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or equiactive prodrugs at equimolar doses plus carbidopa (25 mg kg^{-1} i.p., 1 h before).

Compound	Total activity ± s.e.m., n = 6 (counts)		Relative total activity of prodrug %	Peak activity ± s.e.m., n = 6 (counts)		Relative peak activity of prodrug %
	L-Dopa	Prodrug		L-Dopa	Prodrug	
8. 2-Tetrahydropyranylmethyl	14 794 ±1 679	16 044 ±2 670	108	1414 ± 254	1327 ± 302	94
14. Phenoxyethyl	12 235 ±1 273	13 259 ±1 451	108	1245 ± 143	1067 ± 174	86
6. 2-(1-Methoxy)propyl	15 695 ±1 273	16 680 ±1 517	106	1317 ± 298	1006 ± 150	76
3. Ethyl	13 916 ±1 599	14 682 ±2 320	106	1298 ± 72	1264 ± 207	97
5. 2-Hydroxypropyl	15 541 ±1 605	14 977 ±1 716	96	1207 ± 89	1224 ± 137	101
2. Methyl	16 945 ±1 678	16 069 ±2 299	95	1430 ± 216	1327 ± 155	93

(for example see Fig. 1a). Reversal of reserpine-induced akinesia following administration of the 2-(1-methoxy)propyl (6) ester was prolonged compared with that following the administration of L-dopa itself (Fig. 1b; $P < 0{\cdot}01$).

Intraperitoneal administration of the *p*-methoxyphenylethyl (13), n-propyl (4), phenylethyl (11), *m*-trifluoromethylbenzyl (10) (Fig. 1c), cyclohexyl (7), *p*-chlorophenylethyl (12) and benzyl (9) (Fig. 1d) ester prodrugs (1 mmol kg^{-1} in each case) also

Table 3. Reversal of reserpine-induced akinesia in mice by intraperitoneal administration of L-dopa (1 mmol kg^{-1}) plus carbidopa (25 mg kg^{-} i.p., before) or prodrugs at equimolar doses plus carbidopa (25 mg kg^{-1} i.p., 1 h before).

Compound	Total activity ± s.e.m., n = 6 (counts)		Relative total activity of prodrug %	Peak activity ± s.e.m., n = 6 (counts)		Relative peak activity of prodrug %
	L-Dopa	Prodrug		L-Dopa	Prodrug	
13. *p*-Methoxyphenylethyl	10408 ±1570	8227† ±1954	79	756 ± 158	730 ± 233	97
4. n-Propyl	15012 ±2585	11836* ±3042	79	944 ± 216	957 ± 226	101
11. Phenylethyl	18917 ±2761	14784** ±1399	78	1826 ± 171	1149 ± 204†	63
10. *m*-Trifluoromethylbenzyl	15254 ±999	7301** ±995	48	1379 ± 144	842 ± 172†	61
7. Cyclohexyl	13358 ±1558	5433** ±1498	41	965 ± 125	476 ± 100†	49
12. *p*-Chlorophenylethyl	15541 ±1550	4609** ±1043	30	1349 ± 214	781 ± 305	58
9. Benzyl	10026 ±2412	2336** ±1052	23	802 ± 225	191 ± 144†	24

†$P < 0.05$, *$P < 0.01$, **$P < 0.001$.

reversed reserpine-induced akinesia, but the overall activity was reduced compared with that observed following administration of L-dopa (Table 3).

Reversal of reserpine-induced akinesia in mice following oral administration of L-dopa or prodrugs

Oral administration of L-dopa (1 mmol kg^{-1}) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before) reversed reserpine-induced akinesia and this effect was virtually identical to that produced by intraperitoneal administration of L-dopa (1 mmol kg^{-1}) plus carbidopa (25 mg kg^{-1} i.p., 1 h before). Locomotor activity increased rapidly and was maximal by approximately 1 h but had declined to control values by 4 h.

Oral administration of the prodrugs in equimolar doses to L-dopa plus carbidopa (25 mg kg^{-1} i.p., 1 h before) reversed reserpine-induced akinesia in mice with variable effects (examples are shown in Fig. 2a–d).

Following oral administration of ethyl (3) (1 mmol kg^{-1}) and methyl (2) (1 mmol kg^{-1}; Fig. 2a) ester prodrugs, reserpine-induced akinesia in mice was reversed, but the overall activity was greater than that observed following the administration of L-dopa itself (Table 4) although the time-course did not differ.

The overall activities of the 2-tetrahydropyranylmethyl (8), 2-(1-methoxy)propyl (6), 2-hydroxypropyl (5), n-propyl (4), benzyl (9) and phenoxyethyl esters (14) (1 mmol kg^{-1} in each case) were equal compared with that of L-dopa following oral administration (Table 4). The time-courses following administration of the 2-hydroxypropyl (5), n-propyl (4) and benzyl (9) ester prodrugs were not different compared with that of L-dopa (Fig. 2b for example) although the 2-tetrahydropyranylmethyl (8), 2-(1-methoxy)propyl (6) and phenoxyethyl (14) esters demonstrated a more gradual reduction of locomotor activity (Fig. 2c for example; $P < 0.05$).

Oral administration of the cyclohexyl (7), *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) and *p*-methoxyphenylethyl (13) esters (1 mmol kg^{-1} in each case) also reversed reserpine-induced akinesia in mice, but the overall activity was less than that observed following administration of L-dopa itself (Table 5). The reversal of akinesia following administration of the cyclohexyl (7) ester followed a similar time-course to that observed following L-dopa administration. The *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) and *p*-methoxyphenylethyl (13) esters, however, exhibited different time courses which were characterized by flatter curves of less intensity and later peak effects but with no prolongation of effect ($P < 0.001$; Fig. 2d for example).

The production of circling behaviour in rats with a prior unilateral 6-OHDA lesion of the MFB by L-dopa or prodrugs

Administration of L-dopa (0·25 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) induced a tight contraversive posture and rapid contraversive circling behaviour in rats with a prior unilateral

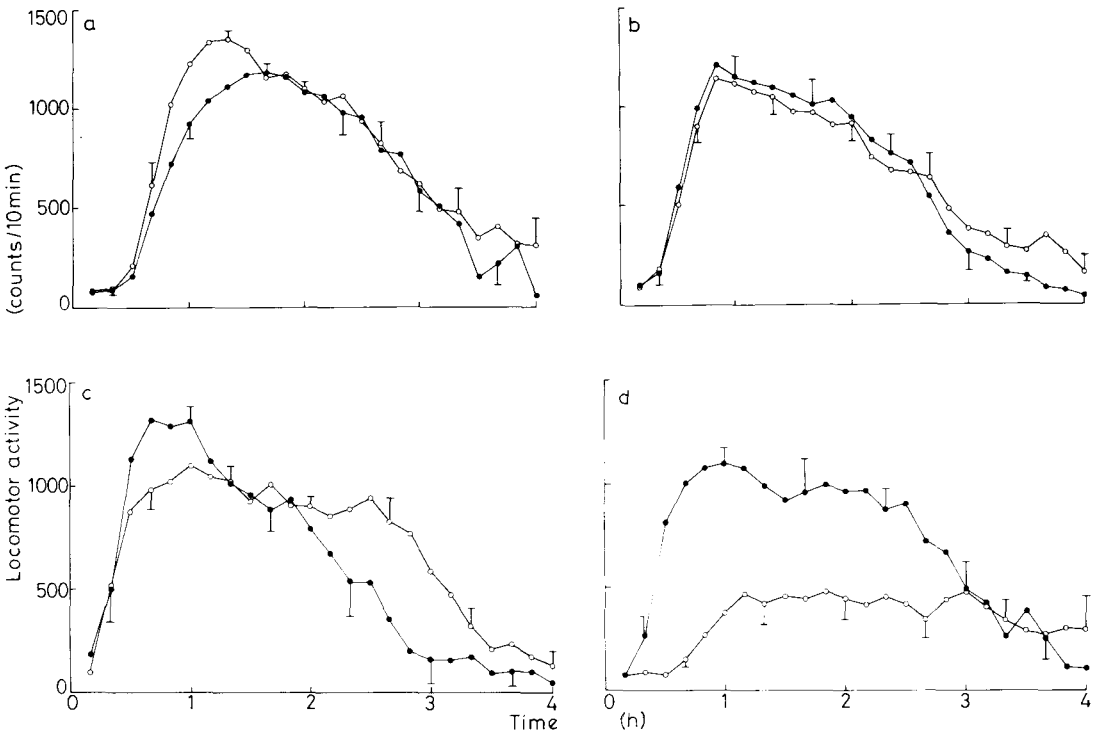


FIG. 2. Reversal of reserpine-induced akinesia in mice following oral administration of equimolar doses of L-dopa (1 mmol kg^{-1}, —●—) and prodrug ester (1 mmol kg^{-1} —○—) (a) methyl (2), (b) 2-hydroxypropyl (5), (c) 2-tetrahydropyranylmethyl (8) and (d) phenylethyl (11) esters. All animals received carbidopa (25 mg kg^{-1} i.p., 1 h before).

Table 4. Reversal of reserpine-induced akinesia in mice following oral administration of equimolar doses of L-dopa (1 mmol kg^{-1}), prodrugs more active than L-dopa (ethyl, methyl) or prodrugs equiactive to L-dopa (2-tetrahydropyranylmethyl, 2-(1-methoxy)propyl, 2-hydroxypropyl, n-propyl, benzyl, phenoxyethyl). All animals received carbidopa (25 mg kg^{-1} i.p., 1 h before).

Compound	Total activity ± s.e.m., n = 6 (counts)		Relative total activity of prodrug %	Peak activity ± s.e.m., n = 6 (counts)		Relative peak activity of prodrug %
	L-Dopa	Prodrug		L-Dopa	Prodrug	
3. Ethyl	12 900 ±726	15 204** ±1 058	118	903 ± 76	938 ± 81	104
8. 2-Tetrahydropyranylmethyl	14 416 ±1 817	16 726 ±925	116	1310 ± 61	1093 ± 53†	83
2. Methyl	15 955 ±1 560	18 183** ±1 117	114	1187 ± 46	1352 ± 39†	114
6. 2-(1-Methoxy)propyl	11 926 ±1 526	12 784 ±1 214	107	1074 ± 96	933 ± 100	87
5. 2-Hydroxypropyl	14 613 ±1 150	15 023 ±1 578	103	1209 ± 199	1143 ± 78	95
4. n-Propyl	14 354 ±2 033	14 301 ±1 411	100	1264 ± 93	1193 ± 82	94
9. Benzyl	14 108 ±2 205	12 973 ±2 682	92	1048 ± 141	1070 ± 188	102
14. Phenoxyethyl	14 469 ±1 101	13 359 ±993	85	1166 ± 92	956 ± 110	82

†$P < 0.05$. **$P < 0.001$.

Table 5. Reversal of reserpine-induced akinesia in mice following oral administration of equimolar doses of L-dopa (1 mmol kg^{-1}) or prodrugs. All animals received carbidopa (25 mg kg^{-1} i.p., 1 h before).

Compound	Total activity ± s.e.m., n = 6 (counts)		Relative total activity %	Peak activity ± s.e.m., n = 6 (counts)		Relative peak activity of prodrug %
	L-Dopa	Prodrug		L-Dopa	Prodrug	
7. Cyclohexyl	11 645 ±1 407	11 020† ±1 219	86	881 ± 124	816 ± 201	93
10. *m*-Trifluoromethylbenzyl	14 795 ±1 189	7 493* ±1 289	51	1167 ± 152	601 ± 144†	51
11. Phenylethyl	16 430 ±1 524	8 213** ±1 894	50	1101 ± 74	478 ± 118*	43
12. *p*-Chlorophenylethyl	15 288 ±1 410	6 790** ±1 785	44	1345 ± 109	578 ± 201*	43
13. *p*-Methoxyphenylethyl	13 149 ±1 398	2 116** ±520	16	1085 ± 75	149 ± 60**	14

†$P < 0.05$, *$P < 0.01$, **$P < 0.001$.

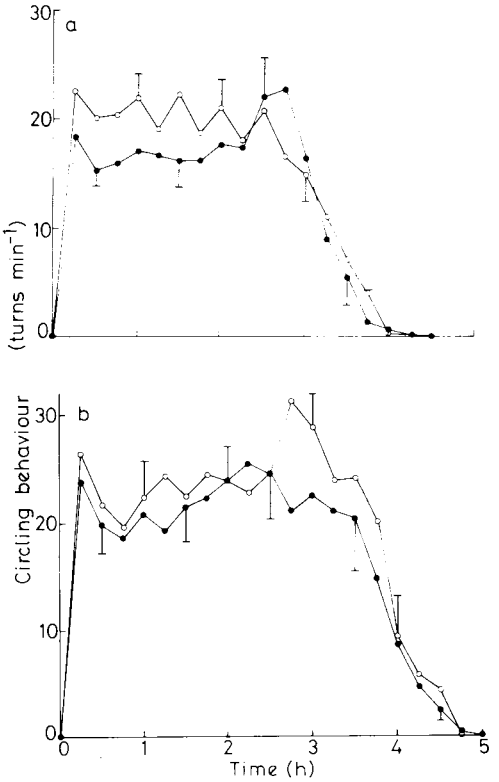


FIG. 3. Circling behaviour in rats following intraperitoneal administration of equimolar doses of L-dopa (1) (0·25 mmol kg^{-1}, —●—) or prodrug ester (0·25 mmol kg^{-1}, —○—) (a) n-propyl (4) and (b) methyl (2) esters. All animals received carbidopa (25 mg kg^{-1} i.p., 1 h before).

6-OHDA lesion of the MFB (Fig. 3). Circling commenced 15 min after L-dopa administration and continued for 3–4 h before declining over approximately 1 h. Intraperitoneal administration of the ester prodrugs in equimolar doses to L-dopa plus carbidopa (25 mg kg^{-1} i.p., 1 h before) induced similar contraversive circling behaviour.

Table 6. Circling behaviour in rats following intraperitoneal administration of equimolar doses of L-dopa (0·25 mmol kg^{-1}) or prodrugs. All animals received carbidopa (25 mg kg^{-1} i.p., 1 h before).

Compound	Total activity ± s.e.m., n = 6–10 (circles)		Relative total activity of prodrug %
	L-Dopa	Prodrug	
4. n-Propyl	226 ± 27	260 ± 13†	115
8. 2-Tetrahydropyranylmethyl	226 ± 27	258 ± 14†	114
2. Methyl	340 ± 23	386 ± 39†	113
3. Ethyl	340 ± 23	383 ± 40†	113
7. Cyclohexyl	310 ± 32	334 ± 33	108
6. 2-(1-Methoxy)propyl	304 ± 36	324 ± 39	107
11. Phenylethyl	304 ± 31	308 ± 31	101
12. *p*-Chlorophenylethyl	304 ± 36	306 ± 39	101
13. *p*-Methoxyphenylethyl	304 ± 31	305 ± 39	100
9. Benzyl	310 ± 32	303 ± 26	98
5. 2-Hydroxypropyl	352 ± 38	336 ± 28	95
14. Phenoxyethyl	352 ± 38	324 ± 42	92
10. *m*-Trifluoromethylbenzyl	360 ± 42	315 ± 33	88

†$P < 0.05$.

Administration of the n-propyl (4), 2-tetrahydropyranylmethyl (8), methyl (2) and ethyl (3) esters (0·25 mmol kg^{-1} in each case) induced circling behaviour in rats with a prior unilateral 6-OHDA lesion of the MFB, but the overall activity was greater than that observed following administration of L-dopa itself (Table 6) although the time-course did not differ (Fig. 3a, b for example).

Circling behaviour induced by the administration of the cyclohexyl (7), 2-(1-methoxy)propyl (6), phenylethyl (11), *p*-chlorophenylethyl (12), *p*-methoxyphenylethyl (13), benzyl (9), 2-hydroxypropyl (5), phenoxyethyl (14) and *m*-trifluoromethylbenzyl (10) esters (0·25 mmol kg^{-1} in each case) did not differ from that observed following administration of L-dopa itself (Table 6) in either intensity or duration.

DISCUSSION

We have compared the ability of L-dopa prodrugs to reverse the akinesia induced in mice by reserpine and to produce circling behaviour in 6-OHDA lesioned rats relative to L-dopa as an index of their potential therapeutic use. In all these experiments animals were pretreated with the peripheral L-aromatic amino acid decarboxylase inhibitor, carbidopa. This procedure was employed since invariably patients with Parkinson's disease are treated with carbidopa to limit peripheral metabolism of L-dopa so making more drug available for conversion to dopamine within brain.

Initially prodrug esters of L-dopa were administered intraperitoneally to reserpinized mice to assess the degree of activity relative to L-dopa as an index of the rate of hydrolysis and subsequent conversion to dopamine. L-Dopa itself was highly effective on intraperitoneal administration in increasing locomotor activity in mice, an effect lasting some 4 h. Ester prodrugs of L-dopa on intraperitoneal administration all caused a reversal of reserpine-induced akinesia indicative of central dopaminergic activity.

However, while some compounds in equimolar amounts were as active as L-dopa overall, no compound was more effective in this model. Indeed, a number of the esters compared were less active than L-dopa. This presumably reflects the rate or degree of conversion to L-dopa and then to dopamine. The only exception was the 2-(1-methoxy)-propyl ester (6) which over the whole time course of action was equiactive to L-dopa but had a more prolonged but less intense effect.

Subsequently, the ester prodrugs were examined following oral administration to assess the degree of bioavailability compared with the intraperitoneal route. After oral administration, the ethyl (3) and methyl (2) ester prodrugs were more active than L-dopa overall, but with the same time courses of action. This suggests greater absorption of these derivatives compared with L-dopa itself. Four ester prodrugs, namely 2-tetrahydropyranylmethyl (8), 2-(1-methoxy) propyl (6), 2-hydroxypropyl (5) and phenoxyethyl (14) were equiactive to L-dopa as had also been the case on intraperitoneal administration. However, two other compounds, namely n-propyl (4) and benzyl (9) esters, equiactive to L-dopa on oral administration were less active intraperitoneally. This suggests that either these compounds are better absorbed than L-dopa on oral administration or that they undergo extensive hydrolysis within the gastrointestinal tract to yield L-dopa itself. Those compounds which were less effective than L-dopa on oral administration, namely cyclohexyl (7), *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) and *p*-methoxyphenylethyl (13) esters, were also less effective on intraperitoneal administration. This suggests that the rate of conversion of these drugs to L-dopa may be rate-limiting and independent of the route of administration.

The reserpinized mouse model mimics Parkinson's disease in terms of the depletion of dopamine. However, the circling rodent model is probably a more appropriate test bed for antiparkinsonian drugs since it involves central destruction of dopamine neurons and the consequential loss of brain dopamine content with development of post-synaptic dopamine receptor supersensitivity. These alterations in brain function are reflected by the lower dose of L-dopa required to produce a clear pharmacological response in these animals.

Interestingly, none of the compounds tested were less active than L-dopa in initiating circling behaviour. Moreover, some compounds, namely n-propyl (4), 2-tetrahydropyranylmethyl (8), methyl (2) and ethyl (3) esters, were more active than L-dopa (1). These results were surprising and unexpected in the light of the findings in the reserpinized mice. We can offer no definitive explanation for these findings. However, this may relate to species difference in the degree of ester hydrolysis and subsequent conversion to dopamine in the rat and mouse. Alternatively, in the rat model the amount of dopamine generated for all of the prodrug esters may be sufficient to maximally stimulate supersensitive dopamine receptors and so produce a marked pharmacological response. In the subsequent paper, we have examined alterations of homovanillic acid and 3,4-dihydroxyphenylacetic acid in the striatum and tuberculum olfactorium of reserpinized mice following intraperitoneal administration of these prodrug esters as an index of brain dopamine metabolism (Cooper et al 1987).

In general, the compounds tested have not demonstrated markedly greater activity or a more sustained effect than L-dopa itself and they would be unlikely to offer major therapeutic advantages on intermittent oral dosage. However, their various physicochemical properties may make them more suitable intravenous or even subcutaneous infusions. Thus, compared with L-dopa, which is a hydrophilic but poorly water-soluble compound, the ester derivatives exhibit high water solubility (Marrel et al 1985a). This would allow solutions of a convenient volume to be used for continuous drug administration strategies. At present the large volumes of

solution required to dissolve L-dopa make the use of infusion technology for parkinsonian patients difficult on a routine basis.

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