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L-Dopa esters as potential prodrugs: effect on brain concentration of dopamine metabolites in reserpinized mice‡

D. R. COOPER, C. MARREL†, H. VAN DE WATERBEEMD†, B. TESTA†, P. JENNER AND C. D. MARSDEN*

MRC Movement Disorders Research Group, University Department of Neurology and Parkinson's Disease Society Research Centre, Institute of Psychiatry and King's College Hospital Medical School, London SE5, UK and †School of Pharmacy, University of Lausanne, CH-1005 Lausanne, Switzerland

The intraperitoneal administration of L-dopa and a series of ester prodrugs of L-dopa to reserpinized mice produced elevations of striatal and tuberculum olfactorium homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels. Differences in the pattern of change produced by individual drugs, compared with L-dopa, were observed. Only the phenoxyethyl ester caused elevations of both striatal and tuberculum olfactorium HVA and DOPAC, greater than those measured following L-dopa administration. Overall the *m*-trifluoromethylbenzyl, phenylethyl, *p*-chlorophenylethyl and *p*-methoxyphenylethyl ester prodrugs produced greater elevations of striatal and tuberculum olfactorium HVA, but not DOPAC, compared with L-dopa. The administration of the 2-tetrahydropyranylmethyl derivative only enhanced striatal HVA and striatal and tuberculum olfactorium DOPAC concentrations. Changes of HVA and DOPAC tissue concentrations following administration of the 2-hydroxypropyl, n-propyl, methyl, ethyl and 2-(1-methoxy)propyl ester prodrugs were comparable with those produced by the administration of L-dopa itself. The alterations in striatal and tuberculum olfactorium HVA and DOPAC levels observed did not correlate with the ability of these compounds to elicit locomotor activity in reserpinized mice.

L-Dopa has inconsistent oral bioavailability and a short half-life in-vivo resulting in wide interdose variation of drug levels. These unfavourable pharmacokinetic properties of the drug may be responsible for some of the problems that occur during chronic L-dopa therapy for Parkinson's disease. One approach to circumvent these problems is to synthesize prodrugs of L-dopa which may be more water- and/or lipid-soluble and may cleave in-vivo to liberate L-dopa. So far, however, possible prodrugs of L-dopa do not appear to have led to agents more useful clinically than L-dopa itself (Hanson & Utley 1965; Lai & Mason 1973; Brossi et al 1974; Felix et al 1974; Bodor et al 1977). We have developed a series of L-dopa derivatives produced by esterification of the carboxy function of L-dopa which may act as prodrugs (Marrel et al 1985a, b).

In a previous paper we reported the ability of the prodrug esters of L-dopa to elicit locomotor activity in mice pretreated with reserpine (Cooper et al 1987). Whilst some of the compounds tested were as active as L-dopa in reversing reserpine-induced akinesia, other compounds were less active. These variable results may be due to different in-vivo hydrolysis rates for the esters. Hydrolysis in-vivo releases L-dopa which, in the presence of a peripheral L-aromatic amino acid decarboxylase inhibitor, would undergo cerebral decarboxylation to dopamine. Subsequent metabolism of dopamine gives rise to homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) which may be used as an index of brain dopamine turnover. Therefore, in the present study we have measured HVA and DOPAC in areas of brain following the administration of L-dopa or prodrugs to rodents to determine whether differences in their effects on locomotor activity and their rates of hydrolysis are reflected by differential changes in dopamine metabolites in dopaminergic brain areas. An initial report on the biochemical effects of the methyl ester of L-dopa has been published elsewhere (Cooper et al 1984).

* Correspondence.

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

MATERIALS AND METHODS

Drugs and reagents

L-Dopa (1) (Roche Products Ltd); ester prodrugs of L-dopa (2–14) as hydrochlorides (Table 1; Marrel et al 1985a, b); reserpine (Halewood Chemicals Ltd); carbidopa (α-methyldopa hydrazine, Merck, Sharp & Dohme Ltd); Sephadex G10 (Pharmacia

Fine Chemicals); BRIJ-35 (30%, Technicon); dopamine hydrochloride, HVA and DOPAC (Sigma Chemical Co. Ltd); perchloric acid, potassium hydroxide, formic acid, disodium hydrogen phosphate dodecahydrate, potassium ferricyanide, ammonia solution (0·88, 33%), cysteine, ethylenediamine and hydrochloric acid (Fisons Scientific Apparatus Ltd).

Determination of striatal and tuberculum olfactorium HVA and DOPAC concentrations in mice

Male mice (20–25 g; Tuck No. 1) were treated with reserpine (5 mg kg^{-1} i.p.; 18–24 h before). On the day of the experiment animals received carbidopa (25 mg kg^{-1} i.p.; suspended in 1% w/v methylcellulose). After 60 min, L-dopa (200 mg kg^{-1} i.p., 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 of 7% w/v $NaHCO_3$) or an equimolar dose of an ester prodrug (1 mmol kg^{-1} i.p.; dissolved in glass-distilled water) or vehicle, was administered. The species of rodent, route of administration and doses were identical to those used in other experiments in which the ability of L-dopa or ester prodrugs to reverse the akinesia induced in mice by reserpine was studied (Cooper et al 1987).

Mice were killed by cervical dislocation and decapitation at 0 h (vehicle only), 0·5, 1·0, 2·0, 4·0 and 6·0 h following L-dopa or ester prodrug administration. Brains were rapidly removed onto ice and the paired striatum and tuberculum olfactorium were dissected as follows. The brain was laid with ventral surface facing upwards and the tuberculum olfactorium was removed between the levels of the olfactory bulbs and the anterior hypothalamus with iris forceps. The brain was then placed with its dorsal surface uppermost and the corpus callosum was divided along the midline. The two hemispheres were teased apart and the exposed corpus striata were dissected out with iris forceps to the level of the white matter. Paired samples were immediately frozen on an ice block and weighed. Tissue samples were homogenized for approximately 5 s in 1·0 mL ice-cold 0·4 M perchloric acid using a 15 mL glass mortar with motorized Teflon pestle (Jencon). Excess perchlorate was precipitated by the addition of 0·05 mL potassium hydroxide-formic acid mixture (24·5 mL 90% HCOOH in 100 mL 10 M KOH). Standards and blanks were prepared by the addition of 0, 100, 200 or 500 ng HVA and DOPAC (dissolved in 0·1 mL 0·4 M $HClO_4$) to a cerebellar homogenate (approximately 50 mg mouse cerebellum in 0·9 mL 0·4 M $HClO_4$).

Homogenates were frozen at −20 °C overnight. On the following day the samples were thawed and centrifuged for 20 min at 4 °C at 10 000*g* using a Mistral 6L centrifuge. The resulting supernatant was decanted onto Bio-Rad Econo columns packed with Sephadex G10 (0·7 × 4 cm) and the eluate discarded. HVA and DOPAC were eluted by the method of Early & Leonard (1978) with a slight modification of the elution pattern to obtain maximum recovery. The columns were eluted with 0·01 M HCl (2 × 1·0 mL, 0·5 mL and 2 × 0·9 mL) followed by 0·005 M $NaHPO_4$ (0·6 mL and 0·5 mL) which were discarded. HVA and DOPAC were recovered by the further addition of 0·005 M Na_2HPO_4 (3 × 0·5 mL) to the columns. The eluates were collected, pooled and placed into sample cups and stored at 4 °C before the determination of HVA and DOPAC by automated fluorimetric analysis using a slight modification of the methods of Westerink & Korf (1976).

Reagents for automated HVA and DOPAC assays

All water used was glass-distilled. A phosphate buffer (0·005 M) was prepared by dissolving 1·79 g of disodium hydrogen phosphate dodecahydrate in 1 L of water. For the HVA assay the ferricyanide reagent contained 10 mg of $K_3Fe(CN)_6$ in a mixture of 85 mL water and 15 mL concentrated ammonia (0·88, 33%). A cysteine solution contained 10 mg of cysteine in 100 mL water to which was added 20 drops of BRIJ-35 (30%) to improve the flow of the reagents. The ethylenediamine (EDA) reagent was prepared by carefully mixing 10 mL (3 × distilled) with 5 mL concentrated HCl and 5 mL of water. Forty drops of BRIJ-35 (30%) were added to this solution to improve the flow characteristics of reagents. A solution of ferricyanide for the DOPAC assay contained 0·2 mg $K_3Fe(CN)_6$ in 100 mL of water.

Determination of HVA concentrations

Sample cups containing the HVA and DOPAC eluates were placed into an automatic sampler (Carlo Erba Distributor SD3). Each sample was collected for 45 s and introduced into a continuous flow system which was powered by a peristaltic pump (Gilson). Flow rates were governed by the internal diameters of the tubings (Sterilin) attached to the pump and each collection was followed by a 45 s washout with phosphate buffer. Samples (1·2 mL min^{-1}) and ferricyanide reagent (0·23 mL min^{-1}) were mixed by passing through a single coil mixer whereupon cysteine solution (0·23 mL min^{-1}) was introduced into the system. A second single coil

mixer ensured thorough mixing of the combined solutions. The resulting mixture was passed through a flow-cell in a Locarte fluorimeter (Model 8–9) fitted with a mercury lamp and fluorescence measured using a primary Kodak Wratten 312 nm filter and secondary Corning C.S.3-73 filter. The fluorimeter was connected to a pen-recorder to produce a trace showing peaks of fluorescence. A fresh solution of HVA (500 ng in 1·5 mL 0·005 M phosphate buffer) was introduced directly into the assay. Peaks produced following assay of this solution were used to estimate absolute recovery and these were compared with 500 ng standard peaks to obtain relative recovery. Tissue contents of HVA ($\mu g\ g^{-1}$) were calculated from a standard curve with corrections for tissue weights and dilution factors.

Determination of DOPAC concentration

The remaining eluates were sampled (as described above) and introduced into a continuous flow system for the DOPAC assay. Samples (0·42 mL min^{-1}) were mixed with ferricyanide reagent (0·8 mL min^{-1}) and EDA reagent by passing through a double coil mixer. The resulting solution passed through a large coil in an oil bath at 73 °C for 13 min and subsequently through the flow-cell in the fluorimeter (Locarte, Model 8–9). Fluorescence was measured using a primary Kodak Wratten 47B filter and secondary Corning C.S.3-69 filter. Calibration of the pen recorder, using a fresh solution of DOPAC (500 ng in 1·5 mL 0·005 M phosphate buffer), estimation of recovery and calculation of tissue contents (DOPAC, $\mu g\ g^{-1}$) were carried out in the same way as for the HVA assay.

Cross-reactivity experiments

Solutions of L-dopa and ester prodrugs (200 ng and 1 μg in 1·5 mL) were introduced into the automated assays for HVA and DOPAC to determine if these substances cross-reacted with HVA or DOPAC assay procedures. In other experiments L-dopa (1 μg) or ester prodrugs (1 μg) were added to cerebellar homogenates (prepared in the manner described above). Following centrifugation and elution of the supernatants the HVA and DOPAC fraction was assayed to determine whether cross-reactivity occurred following column separation. In similar experiments HVA (100 ng) and DOPAC (100 ng) were added to the cerebellar homogenates containing L-dopa (1 μg) or ester prodrugs (1 μg). These samples were treated as above and used to determine whether the fluorescence produced by HVA and DOPAC was altered by the presence of L-dopa or the prodrugs.

Statistical analysis

One experiment was performed to determine the basal levels of HVA and DOPAC (0 h, vehicle only). Each drug was tested at each time-point in three mice on two separate occasions and the results from six animals were pooled. On each occasion mice received prodrugs; other animals were treated with L-dopa in parallel fashion. The tissue content of HVA or DOPAC was expressed as the mean $\mu g\ g^{-1}$ (±1 s.e.m.) at each time-point and an estimation of the overall alterations in tissue levels was made by calculating the area under the curve for the time-course of the experiments (AUC, $\mu g\ g^{-1}$/6 h) using a simple computer program. Statistical analysis was by two-tailed two-way analysis of variance to compare the AUC between L-dopa and the prodrugs, and by two-tailed unpaired Student's *t*-test to compare peak tissue levels of HVA and DOPAC expressed as mean $\mu g\ g^{-1}$ (±1 s.e.m.).

RESULTS

Recovery and cross-reactivity experiments

The recovery of HVA and DOPAC (500 ng) added to cerebellar tissue was 93 ± 2% and 86 ± 2% in a series of 8 experiments. In the range 100–500 ng a linear correlation between standard concentration and peak height was found.

Pilot experiments demonstrated cross-reactivity with L-dopa or ester prodrugs if these were introduced directly into the assays (Table 1). At a

Table 1. Cross-reactivity of L-dopa and ester prodrugs in HVA and DOPAC assays. Solutions containing 200 ng or 1 μg of drug were introduced directly into the assays and expressed as % cross-reactivity where 100% represents peak height produced by 200 ng of HVA and DOPAC standard. Cross-reactivity of >175% represents maximal deflection of the pen-recorder following the introduction of a prodrug into the assay.

	Cross-reactivity (%)		
	HVA assay		DOPAC assay
Compound	200 ng drug	1 μg drug	200 ng drug
1. L-Dopa	5	>175	>175
2. Methyl	4	87	>175
3. Ethyl	4	113	>175
4. n-Propyl	0	138	>175
5. 2-Hydroxypropyl	0	76	>175
6. 2-(1-Methoxy)propyl	32	>175	>175
7. Cyclohexyl	2	>175	>175
8. 2-Tetrahydropyranylmethyl	0	63	>175
9. Benzyl	3	121	>175
10. *m*-Trifluoromethylbenzyl	0	55	>175
11. Phenylethyl	0	75	>175
12. *p*-Chlorophenylethyl	0	50	>175
13. *p*-Methoxyphenylethyl	0	154	>175
14. Phenoxyethyl	0	85	>175

concentration of 200 ng only the 2-(1-methoxy)-propyl ester (6) showed cross-reactivity in the HVA assay. However, at a concentration of 1 μg all compounds tested produced marked cross-reactivity in this assay procedure. In the DOPAC assay procedure an even greater degree of cross-reactivity was observed even at 200 ng of L-dopa (1) of ester prodrug (2–14).

In general, no cross-reactivity was observed if the drugs were first added to cerebellar homogenates and separated by centrifugation and elution of the supernatant on Sephadex-G10 columns. The only exceptions were the benzyl (9) and cyclohexyl (7) esters which cross-reacted in the DOPAC assay procedure. Similarly, prodrugs added to cerebellar tissue did not produce any alteration in fluorescence produced by 100 ng HVA or DOPAC in the same tissue sample (Table 2). The exceptions were the benzyl (9) and cyclohexyl (7) ester prodrugs which potentiated the fluorescence produced by 100 ng DOPAC. No quenching of the peaks was observed. As a result of these pilot experiments the effects of the benzyl (9) and cyclohexyl (7) esters of L-dopa on striatal and tuberculum olfactorium HVA and DOPAC were not studied.

Table 2. Cross-reactivity of L-dopa and ester prodrugs in HVA and DOPAC assays after preparation in cerebellar homogenates, centrifugation and elution of supernatants on Sephadex G10 columns. 1 μg drug plus 100 ng standards were added to homogenate and peak produced was compared with that produced by 100 ng standard alone (100 ng standard = 100%).

Compound	Cross-reactivity (%) of 1 μg + 100 ng standard (100 ng standard only = 100%) HVA	DOPAC
1. L-Dopa	107	93
2. Methyl	108	100
3. Ethyl	105	96
4. n-Propyl	104	89
5. 2-Hydroxypropyl	102	93
6. 2-(1-Methoxy)propyl	93	96
7. Cyclohexyl	94	113*
8. 2-Tetrahydropyranyl-methyl	93	97
9. Benzyl	102	125*
10. *m*-Trifluoromethyl-benzyl	102	93
11. Phenylethyl	107	100
12. *p*-Chlorophenylethyl	105	101
13. *p*-Methoxyphenylethyl	104	105
14. Phenoxyethyl	106	102

* $P < 0.05$.

The effects of reserpine pretreatment and vehicle administration on striatal and tuberculum olfactorium HVA and DOPAC concentrations

Striatal and tuberculum olfactorium HVA and DOPAC concentrations in mice were generally elevated by treatment with reserpine (5 mg kg^{-1}, i.p., 18–24 h before). No differences were found between groups of mice treated with reserpine followed by L-dopa vehicle or prodrug vehicle (Table 3).

Table 3. Striatal and tuberculum olfactorium HVA and DOPAC concentrations in mice. Mice received reserpine (5 mg kg^{-1} i.p., 18–24 h before) or reserpine vehicle (18–24 h before) and L-dopa or prodrug vehicle immediately before killing.

Area and metabolite	Tissue concn (ng g^{-1}) Control and prodrug vehicle	Reserpine and L-dopa vehicle	Reserpine and prodrug vehicle
Striatal HVA	1192 ± 70	2301 ± 317*	2389 ± 190**
Striatal DOPAC	1115 ± 36	1964 ± 216*	1924 ± 122**
Tuberculum olfactorium HVA	726 ± 93	848 ± 66	923 ± 84*
Tuberculum olfactorium DOPAC	661 ± 26	793 ± 45*	812 ± 31*

* $P < 0.05$ compared with control, ** $P < 0.001$ compared with control. No differences were found between the groups treated with reserpine plus L-dopa vehicle or reserpine plus prodrug vehicle.

The effects of administration of L-dopa and of prodrugs on striatal and tuberculum olfactorium HVA and DOPAC concentrations

The administration of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before) produced an elevation of striatal and tuberculum olfactorium HVA and DOPAC concentrations (see for example Fig. 1). Increased tissue levels of all metabolites were observed after 30 min and peak levels normally occurred within 2 h. HVA and DOPAC levels declined over the following 4 h period.

Administration of equimolar doses of prodrugs (1–6; 8; 10–14) plus carbidopa (25 mg kg^{-1} i.p., 18–24 h before) produced similar effects. Thus, all produced elevations of striatal and tuberculum olfactorium HVA and DOPAC concentrations. However, differences in the pattern of change produced by individual drugs, compared with L-dopa, were demonstrated and these are detailed below.

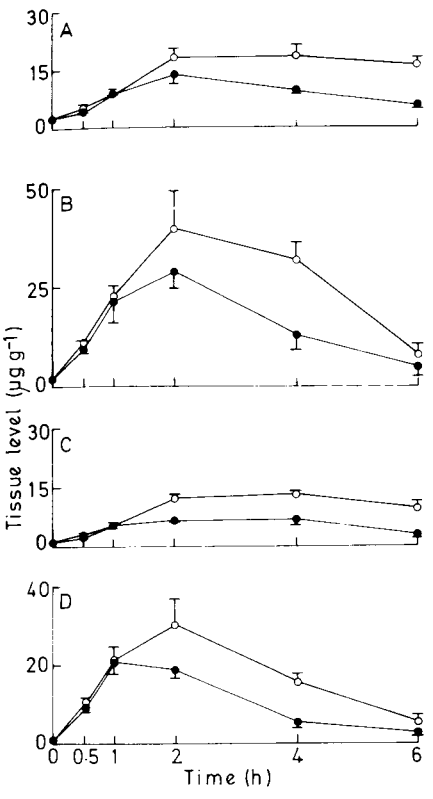

FIG. 1. Alterations in striatal and tuberculum olfactorium HVA and DOPAC concentrations following administration of equimolar doses of L-dopa (1 mmol kg^{-1} i.p. —●—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or the phenoxyethyl ester (14) (1 mmol kg^{-1} i.p. —○—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before). Each value is the mean (±1 s.e.m.) of 6 estimations. A, striatal HVA; B, striatal DOPAC; C, tuberculum olfactorium HVA; D, tuberculum olfactorium DOPAC.

Prodrugs producing a consistent larger increase in striatal and tuberculum olfactorium HVA and DOPAC concentrations compared with L-dopa

Only administration of the phenoxyethyl ester (14) (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to mice treated with reserpine (5 mg kg^{-1} i.p., 18–24 h before) produced elevations of striatal and tuberculum olfactorium HVA and DOPAC concentrations which were greater than those produced by L-dopa itself (Fig. 1). In general, no differences were observed in the initial phase but higher metabolite concentrations were evident over a period of 2–6 h following drug administration. The AUCs were approximately 60–80% greater following administration of the phenoxyethyl (14) ester than following L-dopa (1) administration and this was the only compound to show this pattern of effect (Table 4).

Prodrugs producing a larger increase in either striatal or tuberculum olfactorium HVA or DOPAC concentrations compared with L-dopa

Following intraperitoneal administration of 1 mmol kg^{-1} of the *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) or *p*-methoxyphenylethyl (13) ester prodrugs plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before), elevations of striatal and tuberculum olfactorium HVA were observed which were greater than those produced following administration of an equimolar dose of L-dopa plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to mice treated with reserpine as judged by the relative AUCs (Table 5). These drugs did not consistently elevate striatal or tuberculum olfactorium DOPAC concentrations above levels

Table 4. Area under the curve (AUC, μg g^{-1}/6 h) and peak tissue level (μg g^{-1}) for striatal and tuberculum olfactorium HVA and DOPAC following administration of equimolar doses of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or the phenoxyethyl ester (14) (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before).

Area and metabolite	AUC (μg g^{-1}/6 h) L-Dopa (1)	AUC (μg g^{-1}/6 h) Prodrug (14)	Relative AUC of prodrug (14) (%)	Peak tissue level (μg g^{-1}) L-Dopa (1)	Peak tissue level (μg g^{-1}) Prodrug (14)	Relative peak of prodrug (14) (%)
Striatal HVA	54	87**	162	13·4 ± 2·6	17·6 ± 2·8	131
Striatal DOPAC	94	152*	161	28·6 ± 4·4	39·0 ± 9·7	136
Tuberculum olfactorium HVA	31	57**	183	6·6 ± 1·4	12·0 ± 1·9*	181
Tuberculum olfactorium DOPAC	61	102*	168	20·6 ± 3·0	29·8 ± 6·5	145

* $P < 0·05$, ** $P < 0·001$.

Table 5. Area under the curve (AUC, μg g^{-1}/6 h) and peak tissue level (μg g^{-1}) for striatal and tuberculum olfactorium HVA and DOPAC following administration of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or an equimolar dose of ester prodrug plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before).

Compound	Metabolite	AUC (μg g^{-1}/6 h)	Relative AUC of prodrug		Peak tissue level (μg g^{-1})		Relative peak of prodrug
		L-Dopa	Prodrug	(%)	L-Dopa	Prodrug	(%)
10. *m*-Trifluoromethyl benzyl	Striatal HVA	69	97**	141	17·4 ± 2·1	23·2 ± 2·6	133
	Striatal DOPAC	98	117	119	32·3 ± 4·4	37·8 ± 4·7	117
	Tuberculum olfactorium HVA	44	65**	148	13·2 ± 2·9	16·9 ± 2·7	128
	Tuberculum olfactorium DOPAC	67	107*	158	23·9 ± 2·4	34·3 ± 2·6*	144
11. Phenylethyl	Striatal HVA	58	84**	145	12·3 ± 1·4	19·8 ± 1·6*	161
	Striatal DOPAC	132	192	145	32·7 ± 3·4	46·7 ± 5·8*	143
	Tuberculum olfactorium HVA	34	51**	150	7·8 ± 0·8	11·8 ± 1·5*	151
	Tuberculum olfactorium DOPAC	97	103	106	28·9 ± 0·7	28·1 ± 7·4	97
12. *p*-Chlorophenylethyl	Striatal HVA	58	79**	137	12·3 ± 1·4	28·5 ± 3·0**	232
	Striatal DOPAC	132	177	134	32·7 ± 3·4	44·4 ± 7·3*	136
	Tuberculum olfactorium HVA	34	57**	169	7·8 ± 0·8	23·1 ± 2·1**	296
	Tuberculum olfactorium DOPAC	97	114	117	28·9 ± 0·7	27·1 ± 4·6	94
13. *p*-Methoxyphenylethyl	Striatal HVA	69	99**	144	17·4 ± 2·1	21·3 ± 1·7	122
	Striatal DOPAC	98	99	101	32·3 ± 4·4	24·4 ± 2·0	75
	Tuberculum olfactorium HVA	44	74**	168	13·2 ± 2·9	18·5 ± 2·3*	140
	Tuberculum olfactorium DOPAC	67	96*	142	23·9 ± 2·4	27·5 ± 2·0	115
8. 2-Tetrahydropyranylmethyl	Striatal HVA	57	70*	122	16·7 ± 0·8	17·1 ± 1·1	102
	Striatal DOPAC	104	141*	136	34·9 ± 4·6	41·4 ± 4·9	117
	Tuberculum olfactorium HVA	32	36	115	10·1 ± 0·5	10·9 ± 0·3	99
	Tuberculum olfactorium DOPAC	71	91*	129	26·2 ± 1·8	30·9 ± 2·8	118

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

measured following L-dopa administration. The effect of these ester prodrugs on peak tissue levels of HVA or DOPAC were less consistent (Table 5).

There was no difference in the time-course of increases in the concentration of striatal and tuberculum olfactorium HVA or DOPAC produced by the *m*-trifluoromethylbenzyl (10), phenylethyl (11) and *p*-methoxyphenylethyl (13) esters compared with L-dopa. The *m*-trifluoromethylbenzyl ester (10) is presented as a typical example of this group of drugs (Fig. 2). In contrast, the time-course of effect produced by administration of the *p*-chlorophenylethyl (12) ester prodrug differed from that observed with L-dopa (1). Whilst administration of L-dopa (1) produced maximal elevation of striatal and tuberculum olfactorium HVA after 2 h followed by a gradual decline, the increases observed following administration of the *p*-chlorophenylethyl (12) ester prodrug gradually continued to rise over the 6 h time period (Fig. 3). Also, the elevated levels of striatal and tuberculum olfactorium DOPAC were maintained for longer than observed following administration of L-dopa (1) itself.

Following administration of the 2-tetrahydropyranylmethyl (8) ester (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to mice treated with reserpine (5 mg kg^{-1} i.p., 18–24 h before), striatal HVA and DOPAC, and tuberculum olfactorium DOPAC but not HVA, concentrations were elevated when compared to those measured following L-dopa (1) (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) administration to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before). The time-course of drug effect and the peak brain concentrations of HVA and DOPAC achieved were similar to those observed following L-dopa administration.

Prodrugs producing changes in striatal and tuberculum olfactorium HVA and DOPAC similar to those produced by L-dopa

Administration (1 mmol kg^{-1}, i.p.) of the 2-hydroxypropyl (5), n-propyl (4), methyl (2), ethyl (3) and 2-(1-methoxy)propyl (6) ester prodrugs plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to mice treated with reserpine (5 mg kg^{-1} i.p., 18–24 h before) gradually produced elevations in striatal and tuberculum olfactorium HVA and DOPAC concentrations which were very similar to those observed following administration of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before) (Table 6). The AUCs, peak tissue levels of HVA and DOPAC and time course of effect closely resembled those found following L-dopa administration. The methyl ester (2) prodrug is typical of this group (Fig. 4).

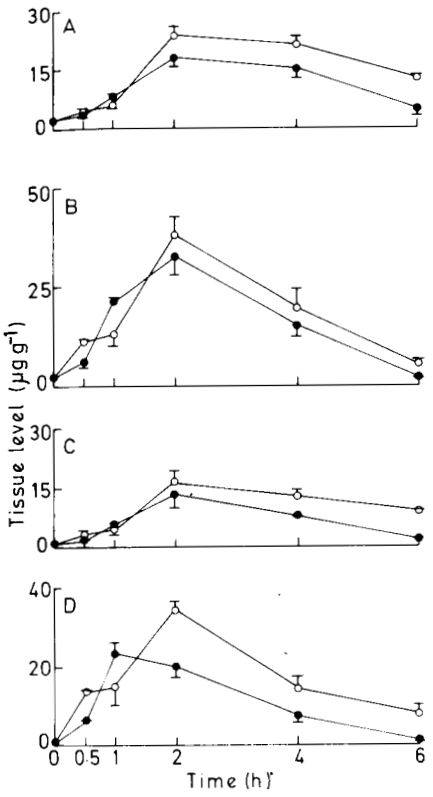

FIG. 2. Alterations in striatal and tuberculum olfactorium HVA and DOPAC concentrations following administration of equimolar doses of L-dopa (1 mmol kg^{-1} i.p. —●—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or the *m*-trifluoromethylbenzyl ester (10) (1 mmol kg^{-1} i.p. —○—) plus carbidopa (15 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before). Each value is the mean (±1 s.e.m.) of 6 estimations. A, B, C, D as in Fig. 1.

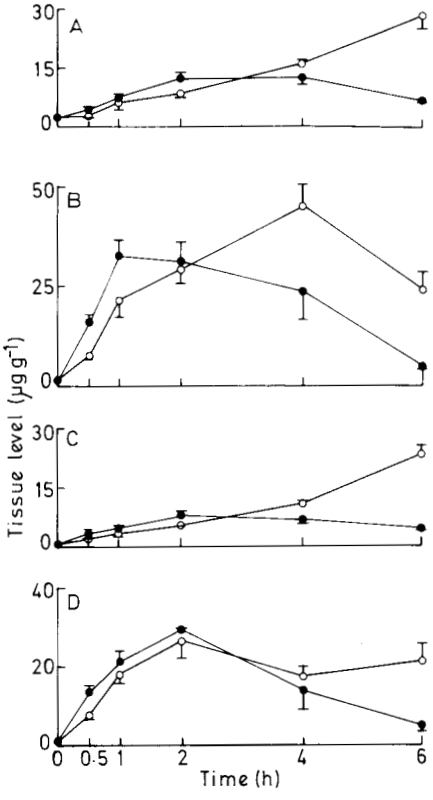

FIG. 3. Alterations in striatal and tuberculum olfactorium HVA and DOPAC concentrations following administration of equimolar doses of L-dopa (1 mmol kg^{-1} i.p. —●—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or the *p*-chlorophenylethyl ester (12) (1 mmol kg^{-1} i.p. —○—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before). Each value is the mean (±1 s.e.m.) of 6 estimations. A, B, C, D as in Fig. 1.

DISCUSSION

We had previously used the reserpinized mouse model to investigate the functional effects of a series of ester prodrugs of L-dopa. Accordingly, we have examined their ability to elevate the levels of the dopamine metabolites HVA and DOPAC in the brains of reserpinized mice. We have previously shown these compounds to differ in their ability to alter motor activity in such reserpine-pretreated animals (Cooper et al 1987) (see Table 7). The results show the ester prodrugs (1–6; 8; 10–14) to cause increases in HVA and DOPAC levels equivalent to those produced by L-dopa.

Reserpine pretreatment results in a general decrease in the brain monoamine content. But despite profound dopamine depletion, increases in the levels of the dopamine metabolites occur, since reserpine treatment causes an increase in the rate of synthesis and of turnover of dopamine. In the present study only the concentrations of HVA and DOPAC were measured as an index of the ability of the prodrugs to alter brain dopamine formation. Dopamine levels themselves could not be measured using the assay procedures employed since L-dopa or the prodrug esters themselves present in brain elute in the same fraction; consequently values for dopamine would have been contaminated.

A further analytical problem is the potential for cross reactivity between L-dopa and the ester prodrugs with the HVA and DOPAC assay procedures. Indeed, all compounds reacted with the reagents used for fluorophore formation and produced flu-

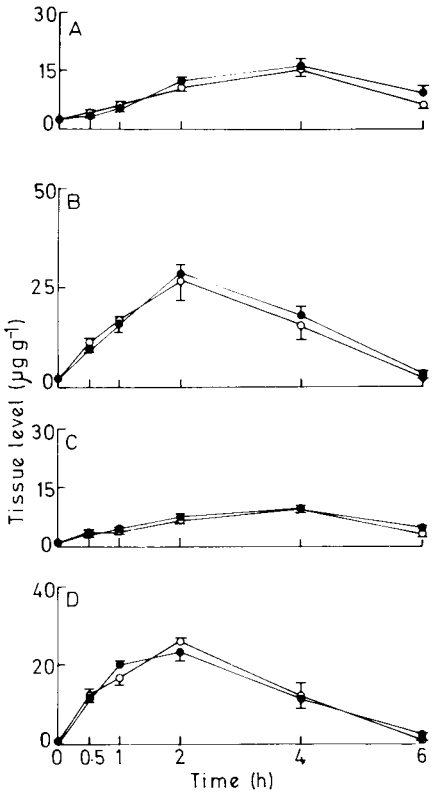

FIG. 4. Alterations in striatal and tuberculum olfactorium HVA and DOPAC concentrations following administration of equimolar doses of L-dopa (1 mmol kg^{-1} i.p., —●—) plus carbidopa (25 mg kg^{-1}, i.p., 1 h before) or the methyl ester (2) (1 mmol kg^{-1} i.p., —○—) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before). Each value is the mean (±1 s.e.m.) of 6 estimations. A, B, C, D as in Fig. 1.

orescent products detectable at the wavelength employed. Consequently it was necessary to determine whether or not the esters eluted from the columns at the same time as the acid metabolites. Fortunately this was not the case for most compounds. Only the benzyl (9) and cyclohexyl (7) prodrugs showed cross reactivity under these circumstances. For this reason these compounds were not considered further.

Following the administration of L-dopa to reserpine-pretreated mice, large increases in the levels of the dopamine metabolites HVA and DOPAC were measured which were maximal between 1–2 h and then gradually declined between 2 h and 6 h. This presumably reflects the penetration of L-dopa into brain with subsequent conversion to dopamine by L-aromatic amino acid decarboxylase and subsequent metabolism of dopamine to HVA and DOPAC by MAO and COMT.

Increases in dopamine metabolite concentrations following the administration of the range of prodrugs of L-dopa were found in all cases. However, three distinct patterns of change were found. Only the phenoxyethyl ester (14) produced elevations of striatal and tuberculum olfactorium HVA and DOPAC concentrations that were greater than those observed following the administration of L-dopa itself.

Another group of compounds produced less consistent increase in striatal or tuberculum olfactorium HVA or DOPAC compared with L-dopa itself. The predominant pattern of change following administration of the *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) and *p*-methoxyphenylethyl (13) esters were increases in striatal and tuberculum olfactorium HVA compared with those observed following L-dopa treatment. In the case of the 2-tetrahydropyranylmethyl (8) ester prodrug, compared with L-dopa, striatal HVA and striatal and tuberculum oflactorium DOPAC were increased. The time-course of effect of these compounds was similar to that observed following L-dopa administration, except in the case of the *p*-chlorophenylethyl ester (12) which produced elevated metabolite levels for a prolonged period.

Administration of a final group of compounds to reserpinized mice produced changes in striatal and tuberculum olfactorium HVA and DOPAC that were similar to those observed following L-dopa treatment. These include the 2-hydroxypropyl (5), n-propyl (4), methyl (2), ethyl (3) and 2-(1-methoxy)-propyl (6) esters. In general, the metabolite concentrations measured, AUCs and time-courses of effect of these prodrug esters closely resembled those observed following administration of L-dopa alone.

Overall these results suggest that all the prodrug esters examined produce at least equivalent changes in brain dopamine formation to those observed following administration of L-dopa itself. However, the results of the behavioural experiments suggest that this is not the case for all compounds (Table 7). The best correlation between production of motor activity and HVA and DOPAC concentrations appeared to be following administration of the 2-hydroxypropyl (5), n-propyl (4), methyl (2), ethyl (3) and 2-(1-methoxy)propyl (6) esters. These compounds were (with the exception of n-propyl) equiactive to L-dopa in producing locomotor activity in reserpinized mice and produced similar changes in

Table 6. Area under the curve (AUC, μg g^{-1}/6 h) and peak tissue level (μg g^{-1}) for striatal and tuberculum olfactorium HVA and DOPAC following administration of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) or an equimolar dose of ester prodrug plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before).

Compound	Metabolite	AUC (μg g^{-1}/6 h)		Relative AUC of prodrug (%)	Peak tissue level (μg g^{-1})		Relative peak of prodrug (%)
		L-Dopa	Prodrug		L-Dopa	Prodrug	
5. 2-Hydroxypropyl	Striatal HVA	54	59	110	13·4 ± 2·6	13·1 ± 1·3	98
	Striatal DOPAC	94	115	122	28·6 ± 4·4	35·7 ± 4·6	125
	Tuberculum olfactorium HVA	31	34	108	6·6 ± 1·4	8·0 ± 0·8	121
	Tuberculum olfactorium DOPAC	61	79*	130	20·6 ± 3·0	25·3 ± 2·6	123
4. n-Propyl	Striatal HVA	57	65	113	16·7 ± 0·8	14·1 ± 1·6	84
	Striatal DOPAC	104	99	95	34·9 ± 4·6	33·6 ± 4·3	96
	Tuberculum olfactorium HVA	32	28*	90	10·1 ± 0·5	7·4 ± 0·3*	73
	Tuberculum olfactorium DOPAC	71	68	96	26·2 ± 1·8	22·6 ± 0·6	86
2. Methyl	Striatal HVA	64	58	91	15·4 ± 1·8	14·9 ± 1·9	97
	Striatal DOPAC	98	91	92	28·2 ± 2·3	26·3 ± 4·7	93
	Tuberculum olfactorium HVA	38	37	95	9·1 ± 0·7	9·1 ± 1·1	100
	Tuberculum olfactorium DOPAC	80	83	103	22·9 ± 2·4	25·8 ± 0·9	113
3. Ethyl	Striatal HVA	64	49*	77	15·4 ± 1·8	12·4 ± 1·4	81
	Striatal DOPAC	98	108	109	28·2 ± 2·3	33·4 ± 4·1	118
	Tuberculum olfactorium HVA	38	31	80	9·1 ± 0·7	7·8 ± 1·3	86
	Tuberculum olfactorium DOPAC	80	72	90	22·9 ± 2·4	23·7 ± 2·6	103
6. 2-(1-Methoxy)propyl	Striatal HVA	54	46	86	12·6 ± 1·7	10·3 ± 1·4	82
	Striatal DOPAC	128	104	81	38·3 ± 2·1	30·3 ± 2·8*	79
	Tuberculum olfactorium HVA	30	27	89	6·6 ± 0·9	6·1 ± 0·8	92
	Tuberculum olfactorium DOPAC	86	82	96	28·6 ± 1·1	28·3 ± 1·0	99

* $P < 0.05$.

Table 7. Relative effect on locomotor activity and on striatal dopamine metabolite concentrations produced by the administration of prodrug esters of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to reserpinized mice (5 mg kg^{-1} i.p., 18–24 h before) compared with those observed following the administration of L-dopa (1 mmol kg^{-1} i.p.) plus carbidopa (25 mg kg^{-1} i.p., 1 h before) to mice treated with reserpine (5 mg kg^{-1} i.p., 18–24 h before).

Compound	% Activity†	% AUC	
		HVA	DOPAC
14. Phenoxyethyl	108	162**	161*
10. *m*-Trifluoromethylbenzyl	48*	141**	119
11. Phenylethyl	78*	145**	145
12. *p*-Chlorophenylethyl	30*	137**	134
13. *p*-Methoxyphenylethyl	79*	144**	101
8. 2-Tetrahydropyranylmethyl	108	122*	136*
5. 2-Hydroxypropyl	96	110	122
4. n-Propyl	79*	113	95
2. Methyl	95	91	92
3. Ethyl	106	77*	109
6. 2-(1-Methoxy)propyl	106	86	81

* $P < 0.05$, ** $P < 0.001$.
† Data from Cooper et al (1987).

striatal and tuberculum olfactorium HVA and DOPAC concentrations when compared with L-dopa.

The phenoxyethyl (14) and 2-tetrahydropyranylmethyl (8) ester prodrugs were as active as L-dopa in producing locomotor activity in reserpinized mice, but overall brain dopamine metabolite concentrations were greater following administration of these compounds than following L-dopa treatment.

The most surprising compounds were the *m*-trifluoromethylbenzyl (10), phenylethyl (11), *p*-chlorophenylethyl (12) and *p*-methoxyphenylethyl (13) ester prodrugs. All these four compounds produced less locomotor activity in reserpinized mice than L-dopa, yet produced overall greater changes in metabolite concentrations in brain. It is not immediately apparent why this should be. Certainly the behavioural effects produced by L-dopa and the prodrugs were identical.

There are a number of possibilities to explain this. It is possible that the prodrugs or 'pro' moiety may interfere with the egress of HVA and DOPAC from brain in a fashion similar to probenecid. However, we have no evidence to support this. Alternatively, these drugs might displace dopamine from dopaminergic neurons, resulting in increased dopamine extraneuronally, but without functional effects. It is also possible that larger amounts of dopamine are formed from these compounds than from L-dopa itself, but that the dopamine formed is non-functional. This might be the case if the prodrugs were converted to dopamine extraneuronally where it would be rapidly metabolized. Indeed prodrug administration consistently caused a greater increase in brain HVA content than in DOPAC levels compared with that observed following administration of L-dopa itself. Since DOPAC is considered to be formed intraneuronally and HVA extraneuro-

nally this would be consistent with the 'non-functional' metabolism of prodrugs at extraneuronal sites. Indeed the prodrugs might be taken up into other neurons containing decarboxylase. If this occurred, for example in 5-HT neurons, dopamine formed would be relatively non-functional.

Another possibility for the apparent lack of correlation between behavioural effects of the prodrugs and the biochemical changes observed might relate to the brain areas studied. In particular, the nucleus accumbens is strongly associated with the production of locomotor activity in rodent species. The nucleus accumbens was not utilized since the assay employed was not sufficiently sensitive to detect HVA or DOPAC in the small amounts of tissue obtained from mice. However, it is unlikely that any better correlation could have been obtained than in the striatum or tuberculum olfactorium. The wide spread distribution of esterase and L-aromatic amino acid decarboxylase in brain results in the extensive formation and metabolism of dopamine in a wide variety of structures. The overall effect is to swamp the brain with L-dopa, dopamine and its metabolites in a non-functional form. Again this would appear to provide the most reasonable explanation for the lack of correlation between motor activity and dopamine metabolite formation and one which would apply to any brain area studied.

Clearly, the behavioural activity of prodrugs of L-dopa cannot be predicted from the production of central dopamine metabolites alone. For seven of the compounds tested we found little disparity and they appear to be hydrolysed and converted to L-dopa and produce behavioural effects comparable with those of L-dopa itself. The four remaining drugs demonstrated little correlation between their behavioural effects and brain dopamine metabolite concentrations, confirming the importance of studying functional models of activity at an early stage in the evaluation of centrally active compounds.

Acknowledgements

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