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The effect of benserazide on the peripheral and central distribution and metabolism of levodopa after acute and chronic administration in the rat

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1 The effects of levodopa alone (50 mg kg^{-1}) and levodopa (10 mg kg^{-1}) plus benserazide (50 mg kg^{-1}) were tested on the levels of dopa, dopamine, 3-methoxytyrosine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), measured by h.p.l.c. with electrochemical detection, in samples of plasma, CSF, urine, striatum and hypothalamus of rats taken 30 min after injection. Levodopa plus benserazide produced significantly higher levels of dopa in plasma and brain than levodopa alone and reduced the peripheral synthesis and metabolism of dopamine.

2 When given chronically over 6 weeks the advantages of adding benserazide (50 mg kg^{-1} day^{-1}) to levodopa (40 mg kg^{-1} day^{-1}) were less marked and although more dopamine was present in the striatum than with levodopa given alone (200 mg kg^{-1} day^{-1}) there was no evidence of any increase in its metabolites (HVA and DOPAC) and therefore of its turnover and utilisation.

3 The most striking effect of chronic treatment with levodopa plus benserazide was the appearance of large quantities of 3-MT in plasma, CSF and brain.

4 When levodopa alone, or levodopa plus benserazide, was given as an acute challenge to animals receiving the same treatment chronically, it was found that levodopa alone still produced increases in striatal dopamine, DOPAC and HVA in those animals dosed chronically on levodopa, but it was less effective in this respect when given with benserazide to the animals dosed with levadopa plus benserazide.

5 It is concluded that this difference in levodopa distribution may depend on the persistence in benserazide-treated animals of 3-MT, which has a long half-life and may compete with dopa for transport into the blood and brain.

6 The implication of these findings to the treatment of Parkinsonism is discussed.

Introduction

Most systemically administered levodopa (L-dopa) is decarboxylated by dopa decarboxylase (L-aromatic amino acid decarboxylase) to dopamine before it can enter the central nervous system. It is also a weak substrate for monoamine oxidase (MAO) but readily metabolised by catechol-*O*-methyl transferase (COMT). When used in the treatment of Parkinsonism it is routinely given with drugs like carbidopa or benserazide which block its decarboxylation peripherally, but do not easily cross the blood brain barrier to affect its conversion to dopamine centrally. These extracerebral dopa decarboxylase inhibitors (ExCDDIs) not only allow smaller doses of L-dopa to be given but reduce any side-effects (e.g. vomiting) that arise from the peripheral actions of dopamine (see Pinder *et al.*, 1976).

One consequence of the use of EXCDDIs is that L-dopa is predominantly metabolised by COMT to 3-methoxy tyrosine (3-methoxy 4-hydroxy phenylalanine, 3-MT or 3-O-methyldopa, 3-MD). Since this metabolite appears to compete with L-dopa for entry into the central nervous system (Gervas *et al.*, 1983) it is important to know how ExCDDIs influence the distribution of levodopa and its metabolites. We have therefore studied the effect of benserazide on the levels of dopa, dopamine and 3-MT in the blood, urine and CSF, as well as the striatum and hypothalamus of rats after its acute administration with levodopa. Levels of the major metabolites of dopamine, e.g. 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were also monitored as an index of the turnover (and function) of dopamine.

The half-life of the metabolite 3-MT (15 h) is also much longer (Kuruma *et al.*, 1970) than that of L-dopa (1 h) and so it is possible that its effects on L-dopa distribution and metabolism may increase with repeated dosing. Consequently, we have given L-dopa alone and with benserazide chronically to rats over six weeks to determine the effects of such dosing on the levels of dopa and dopamine and their metabolites in the blood, CSF, and brain. Since Melamed *et al.* (1983) found that the striatal accumulation of dopamine after injecting L-dopa into rats treated previously for thirty days with L-dopa and cardidopa, was less than when given to untreated animals we have also challenged some of our chronically-treated animals with the dose of L-dopa (with or without benserazide) used acutely. However, in order to obtain a complete picture of the effect of chronic dosing, we again monitored levels not only of dopa and dopamine but of their metabolites in blood, CSF and brain.

Methods

All experiments were performed on male albino rats (University College London, Medical Sciences Animal House – stock) weighing approximately 200 g at commencement. They were divided into six groups assigned the following treatments. (1) Acute L-dopa: a single injection of 50 mg kg^{-1} i.p. 30 min before death. (2) Acute L-dopa with benserazide: these animals were given benserazide, 50 mg kg^{-1} i.p., 30 min before L-dopa 10 mg kg^{-1} and were killed 30 min after that. (3) Chronic L-dopa: L-dopa was given in the drinking water (1.67 g l^{-1}) to achieve a consumption, based on known drinking habits, of approximately 200 mg kg^{-1} day^{-1} over six weeks. They were deprived of this solution 1 h before death. (4) Chronic L-dopa plus acute L-dopa: as for group 3, but given a single i.p. injection of L-dopa (50 mg kg^{-1} as in 1) 30 min before death. (5) Chronic L-dopa with benserazide: these animals were given L-dopa (0.33 g l^{-1}) and benserazide (0.42 g l^{-1}) in their drinking water to achieve doses of approximately 40 mg kg^{-1} day^{-1} and 50 mg kg^{-1} day^{-1} respectively for six weeks. Fluid was removed 1 h before death. (6)

Chronic L-dopa with benserazide plus acute L-dopa with benserazide: as for group 5 but with a single injection of L-dopa 10 mg kg^{-1} plus benserazide 50 mg kg^{-1} i.p 30 min before death.

Animals were deeply anaesthetized with halothane (1%–2% in 95% O_2, 5% CO_2) and the cisterna magna exposed. CSF (approx. 100 μl) was withdrawn into a hypodermic syringe after the dura had been punctured. Anaesthesia was then increased until respiration ceased when the heart was immediately exposed and blood (500 μl) obtained by cardiac puncture. The brain was then perfused through the aorta with ice-cold saline containing ascorbic acid (200 μg l^{-1}) and quickly removed. The striatum and hypothalamus were quickly dissected out and frozen. Urine samples were obtained by puncture of the bladder with a hypodermic needle and syringe. Blood was immediately centrifuged to obtain plasma and the protein precipitated with 20 μl of 60% perchloric acid before further centrifugation (100 g for 5 min); urine was treated similarly. Brain samples were homogenised in 10 vol 0.5 M perchloric acid and centrifuged. All supernatants were frozen to await assay. Concentrations were expressed as pmol mg^{-1} tissue for brain samples, and in μM for blood, CSF and urine. No attempt was made to determine total excretion rate. Recoveries from plasma (data not corrected) ranged from 80% for HVA to 103% for dopa.

Estimations were performed by reverse-phase h.p.l.c. with electrochemical detection. The injection of samples onto the analytical column (150 × 4 mm, Spherisorb ODS-2 5 μm) through a 20 μl loop and pre-column (Upchurch uptight, lichroprep RP18 Anachem) was controlled by a Kontron autosampler MSI 660. Amines were detected (sensitivity 1.5 pmol) at a potential of 0.65 V with an LCA 15 detecter (EDT Research Ld.) and the results analysed on a Schimadzu CRIB integrator (Dyson Instruments). Standard solutions containing known amounts of the five substances being studied were run at two or three different concentrations before the start of each experiment to calibrate the integrator, and representative standards were included routinely after every 5–6 samples to test reliability. The mobile phase which gave the best separation of the amines and metabolites being studied contained the following substances (mmol l^{-1}): citric acid (60), disodium hydrogen phosphate (40), sodium heptane sulphonate (1.0), Na_2 EDTA (0.054) and acetonitrile (380). This was adjusted to pH 2.20–2.25 with HCl. The solvent was degassed (ERMA, ERC3510) and pumped (RR065, HPLC Technology) under 1000 p.s.i. at a rate of 1 ml min^{-1}.

Data were compared by Student's t test for the following levels of significance, * or † at 0.05, ** or †† at 0.01 and *** or ††† at 0.001.

All chemicals were Analar h.p.l.c. grade and prepared in deionised, carbon-swept water, whilst the amines required for standardization of the chromatography were obtained from Sigma Ltd, as was L-dopa. Benserazide was generously donated by Roche Products Ltd., U.K.

Results

Acute administration of L-dopa alone and with benserazide (Table 1)

Neither dopa, dopamine nor any of their metabolites were detected in the plasma or CSF of control animals although, with the exception of dopamine, all were present in both body fluids 30 min after a single injection of levodopa (50 mg kg^{-1} i.p.). Even then no dopa was found in the striatum or hypothalamus, although the increase in DOPAC and HVA in the hypothalamus could indicate its metabolism there.

When a lower dose of L-dopa (10 mg kg^{-1}) was given, but 30 min after benserazide (50 mg kg^{-1}), there was a significant ($P < 0.001$) increase in plasma dopa (2.41 ± 0.73 to 24.7 ± 3.99 μM) in comparison with L-dopa alone. A significant ($P < 0.01$) decrease in the metabolites of dopamine, i.e. DOPAC (2.51 ± 0.45 to 0.82 ± 0.25 μM) and HVA

Table 1 The effect of the acute intraperitoneal injection of L-dopa (50 mg kg^{-1}) or L-dopa (10 mg kg^{-1}) plus benserazide (50 mg kg^{-1}) on the levels of dopa, 3-methoxytyrosine (3-MT), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in plasma, CSF, urine, striatum and hypothalamus of rats

	Dopa	*3-MT*	*DA*	*DOPAC*	*HVA*
Plasma (μM)					
Untreated	0.00	0.00	0.00	0.00	0.00
L-Dopa	2.41 ± 0.73	2.36 ± 0.70	0.00	2.51 ± 0.45	4.19 ± 0.44
L-Dopa + benserazide	24.7 ± 3.99***	4.95 ± 0.76*	0.00	0.82 ± 0.25*	1.11 ± 0.57** †
CSF (μM)					
Untreated	0.00	0.00	0.00	0.00	0.00
L-Dopa	0.95 ± 0.43	0.37 ± 0.14	0.00	1.24 ± 0.58	0.92 ± 0.41
L-Dopa + benserazide	1.38 ± 0.32	1.71 ± 0.21*	0.00	0.31 ± 0.06	0.48 ± 0.11
Striatum (pmol mg^{-1})					
Untreated	0.00	0.00	25.5 ± 0.9	13.6 ± 1.41	4.95 ± 0.57
L-Dopa	0.00	3.25 ± 0.94	20.9 ± 3.30	14.8 ± 2.75	6.95 ± 1.43
L-Dopa + benserazide	11.9 ± 1.5	12.4 ± 1.27***	39.4 ± 1.90***	18.3 ± 1.27	7.45 ± 0.53
Hypothalamus (pmol mg^{-1})					
Untreated	0.00	0.00	2.15 ± 0.5	0.56 ± 0.16	0.00
L-Dopa	0.00	1.34 ± 0.24	3.16 ± 0.61	1.77 ± 0.24††	0.89 ± 0.17
L-Dopa + benserazide	20.1 ± 4.53	11.4 ± 1.26***	5.25 ± 0.71	3.05 ± 0.32	1.03 ± 0.11
Urine (μM)					
Untreated	5.35 ± 3.45	0.00	0.00	6.85 ± 2.90	43.8 ± 13.2
L-Dopa	10.7 ± 3.45	0.00	0.00	312 ± 15.2††	486 ± 33.5††
L-Dopa + benserazide	455 ± 90.5	0.00	0.00	48.3 ± 12.4**	39.9 ± 25.7**

Mean values ± s.e., $n = 6$. Samples 30 min after dosing.
* Significantly different from L-dopa alone.
† Significantly different from untreated control.

(4.19 ± 0.44 to $1.11 \pm 0.57\,\mu$M) was found, but 3-MT was increased ($P < 0.05$). A similar pattern of changes was seen in the CSF, although only the increase in 3-MT was significant ($P < 0.05$).

The most striking feature of adding benserazide to L-dopa was the appearance of dopa in the striatum and hypothalamus, together with significant increases ($P < 0.001$) of 3-MT in both and of dopamine in the striatum.

Analysis of urine samples showed no excretion of 3-MT following either drug treatment. L-Dopa alone was followed by some increased excretion of dopa and a significant rise ($P < 0.01$) in HVA and DOPAC. The addition of benserazide produced a significant increase ($P < 0.01$) in dopa but a significant decrease ($P < 0.01$) in HVA and DOPAC excretion.

Chronic administration of L-dopa alone and with benserazide (Table 2)

The dose of L-dopa chosen for chronic administration (approx $200\,\text{mg kg}^{-1}\,\text{day}^{-1}$) only just produced measurable plasma levels but, despite the lower levels of dopa, dopamine, DOPAC and HVA compared with those found after the acute administration of L-dopa (Table 1), the level of 3-MT was higher. Since these measurements were made 60 min after the drinking bottle containing L-dopa had been withdrawn and an unknown time after the last ingestion of an unknown amount of L-dopa by the animals, the plasma values cannot be compared directly (statistically) with those obtained 30 min after acute administration of L-dopa. Despite this fact the 3-MT:dopa ratio rose from unity after acute L-dopa ($2.36:2.41\,\mu$M), to 49 after chronic dosing ($4.42:0.09\,\mu$M). In CSF the ratios were 8 (acute dosing) and 21 (chronic).

This time factor may be less important when considering urine and brain samples where storage can occur. Thus, despite the very low levels of plasma dopa in the chronically-treated animals it had accumulated in both the striatum and hypothalamus and there was a significant ($P < 0.001$) increase in dopamine in the striatum. 3-MT was also higher in the brain than after acute L-dopa. That the consumption of L-dopa may have been adequate in the chronically-treated animals, despite the low plasma levels at the time of testing, was also confirmed by a significant ($P < 0.001$) increase in its excretion ($667 \pm 172\,\mu$M) compared with that ($10.7 \pm 3.45\,\mu$M) found in acutely injected animals, coupled with the appearance of 3-MT in urine and significantly more DOPAC ($P < 0.01$).

When L-dopa was given chronically with benserazide there was significantly more dopa in plasma ($P < 0.01$) and CSF ($P < 0.01$) but not in the striatum and hypothalamus, in comparison with levodopa alone. The combined dosage increased striatal dopamine ($P < 0.001$) but there were no changes in the concentration of DOPAC and HVA. Dramatic increases in 3-MT were seen in all samples, but especially in the striatum and hypothalamus.

Despite a reduced conversion of dopa to dopamine in the periphery after benserazide it did not increase the excretion of L-dopa, although the urine concentration of 3-MT rose significantly ($P < 0.001$) as did that of HVA ($P < 0.001$).

Acute challenge with L-dopa and L-dopa plus benserazide after their chronic administration (Table 3)

Direct comparison of the levels of dopa, dopamine and their metabolites in chronically-treated animals 30 min after acute challenge with either L-dopa alone or L-dopa plus benserazide, with those found in chronically-treated but unchallenged animals (Table 2), shows the expected increase in plasma dopa after L-dopa with benserazide, but the explained scatter of results negated its significance in animals given L-dopa only. There were also increases in DOPAC and HVA, especially after L-dopa alone.

Changes in amine levels produced by the extra acute injection and a comparison of these values with the changes produced by the same injection in naïve untreated animals, were

Table 2 The effect of chronic L-dopa (approx. $200\,\text{mg kg}^{-1}$) or L-dopa (approx. $40\,\text{mg kg}^{-1}$) plus benserazide ($50\,\text{mg kg}^{-1}$) each day for six weeks on dopa, 3-methoxytyrosine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and dopamine (DA) in the plasma, CSF, urine, striatum and hypothalamus of rats

	Dopa	*3-MT*	*DA*	*DOPAC*	*HVA*
Plasma (μM)					
Untreated	0.00	0.00	0.00	0.00	0.00
L-Dopa	0.09 ± 0.06	4.42 ± 0.55	0.00	0.50 ± 0.18	1.09 ± 0.26
L-Dopa + benserazide	5.67 ± 0.85**	47.8 ± 15.55**	0.00	0.28 ± 0.21	1.32 ± 0.18
CSF (μM)					
Untreated	0.00	0.00	0.00	0.00	0.00
L-Dopa	0.07 ± 0.07	0.58 ± 0.31	0.00	0.08 ± 0.06	0.11 ± 0.07
L-Dopa + benserazide	0.30 ± 0.04*	6.46 ± 0.85***	0.00	0.06 ± 0.03	0.28 ± 0.08
Striatum (pmol mg^{-1})					
Untreated	0.00	0.00	25.5 ± 0.9	13.6 ± 1.41	4.95 ± 0.57
L-Dopa	9.25 ± 1.50	7.65 ± 0.98	34.9 ± 2.45†††	16.9 ± 2.49	7.80 ± 0.84††
L-Dopa + benserazide	9.42 ± 3.35	55.0 ± 3.39***	56.3 ± 2.65***	16.9 ± 1.60	8.85 ± 1.00
Hypothalamus (pmol mg^{-1})					
Untreated	0.00	0.00	2.15 ± 0.51	0.56 ± 0.16	0.00
L-Dopa	1.77 ± 0.49	3.59 ± 1.13	3.11 ± 0.71	1.14 ± 0.39	0.35 ± 0.23
L-Dopa + benserazide	3.83 ± 0.96	53.2 ± 4.42***	4.13 ± 0.49	1.03 ± 0.23	0.29 ± 0.24
Urine (μM)					
Untreaded	5.35 ± 3.45	0.00	0.00	6.85 ± 2.90	43.8 ± 13.2
L-Dopa	677 ± 172†††	24.5 ± 6.20	0.00	51.6 ± 6.45†††	57.5 ± 4.85
L-Dopa + benserazide	559 ± 72	149 ± 14.1***	0.00	71.5 ± 31.2	487 ± 55***

Mean values ± s.e., $n = 6$.
* Significantly different from L-dopa alone.
† Significantly different from untreated control.

Table 3 The effects of acute L-dopa or L-dopa plus benserazide (see Table 1 for doses) given as a challenge to chronically-treated animals

	Dopa	*3-MT*	*DOPAC*	*HVA*	*DA*
Plasma (μM)					
L-Dopa	8.73 ± 6.05	5.46 ± 0.41	3.11 ± 0.51**	8.01 ± 3.54*	—
	(8.64)	(1.04)	(2.61)	(6.92)	
	[+6.23]	[−1.32]	[+0.1]	[2.73]	
L-Dopa + benserazide	16.3 ± 6.3***	62.6 ± 12.8	1.56 ± 0.44***	1.99 ± 0.91	—
	(10.63)	(14.8)	(1.28)	(0.67)	
	[−14.44]	[+9.85]	[+0.46]	[−0.43]	
CSF (μM)					
L-Dopa	0.71 ± 0.45	0.68 ± 0.30	0.15 ± 0.1	0.34 ± 0.19	—
	(0.64)	(0.10)	(0.07)	(0.23)	
	[−0.31]	[−0.27]	[−1.17]	[−0.69]	
L-Dopa + benserazide	0.60 ± 0.04	6.26 ± 0.77	0.31 ± 0.04*	0.74 ± 0.17*	—
	(0.30)	(−0.2)	(0.25)	(0.46)	
	[−1.08]	[−1.91]	[−0.06]	[−0.02]	
Striatum (pmol mg^{-1})					
L-Dopa	10.5 ± 2.05	9.99 ± 1.45	23.4 ± 2.08*	15.6 ± 3.07	39.6 ± 2.15
	(1.25)	(2.34)	(6.5)	(7.8)	(4.7)
	[+1.25]	[−0.91]	[+5.3]	[5.85]	[+9.3]
L-Dopa benserazide	4.79 ± 0.45	60.7 ± 3.89	16.9 ± 1.45	10.8 ± 0.66	55.3 ± 3.32
	(−4.63)	(5.7)	(0.00)	(1.95)	(−1.0)
	[−15.53]	[−6.7]	[−4.9]	[−0.55]	[−14.9]

The drugs were given one hour after withdrawal of drinking fluid used for chronic treatment (see Table 2 for doses). Values for dopa, 3-MT, DOPAC, HVA, and dopamine in plasma, CSF and striatum are for samples taken 30 min after dosing. Mean values ± s.e., $n = 6$. Figures in round parentheses show the absolute increase (− indicates decrease) in amine concentrations proudced by the acute administration of L-dopa alone or with benserazide over those in chronically-treated animals found without further dosing (Table 2). Values in square parentheses give the differences produced (+ increase, − decrease) in levels compared with that obtained when naive animals were given either L-dopa alone or L-dopa plus benserazide (Table 1). * Significantly different from values obtained in chronically-treated animals only (Table 2). For abbreviations used see Tables 1 and 2.

calculated. Values for the first of these measures (found by determining the difference between the absolute values in Tables 2 and 3) are shown in the round parentheses in Table 3. The difference between these values and those for the effects of acute administration in naïve animals (Table 1), which gives an indication of whether or not chronic administration has increased or decreased the metabolism of a subsequent dose, are shown in square parentheses in Table 3. Thus a single injection of L-dopa after chronic treatment gave a plasma level for 3-MT of 5.46 ± 0.4 μM which is (1.04 μM) more than that after chronic dosing alone, but it is a smaller increase and therefore negative [−1.32] with respect to that produced by the same injection of L-dopa into naïve animals (2.36 ± 0.70 μM in Table 1). Since these differences were between means for which there are no individual values (the differences could not be measured in the same animal), they have not been subjected to statistical analysis. Nevertheless, the effects of a single injection of L-dopa do not appear to be modified greatly by its previous chronic administration. The increases in CSF amine levels were all slightly smaller than after the first injection, but striatal dopamine was increased more as was DOPAC and HVA formation. There appeared to be no extra diversion to 3-MT.

By contrast when L-dopa was given with benserazide to animals who had been dosed chronically with the same combinations, shunting of L-dopa to 3-MT [+9.85 μM] was more marked in the plasma and less dopa reached the striatum [15.53 μM] to be converted to dopamine [−14.9 μM] than when given to naïve animals.

Discussion

In this study, we have monitored the levels of dopa, dopamine and three metabolites, DOPAC, HVA and 3-O-methyltyrosine (3-MT) in samples of plasma, urine, CSF, striatum and hypothalamus, to follow the metabolic fate and distribution of L-dopa injected alone or with benserazide, an extra cerebral dopa decarboxylase inhibitor (ExCDDI). The doses of L-dopa and benserazide chosen for this study were not directly related to those used clinically. Benserazide was given in a dose (50 mg kg^{-1} acutely or daily in the chronic studies) which should have ensured blockage of peripheral but not central dopa decarboxylase activity (Bartholini *et al.*, 1967) and so give a clear picture of its effect on L-dopa kinetics. The main findings of these studies are summarised in Figure 1.

There can be no doubt that benserazide given acutely with L-dopa inhibited its decarboxylation, since the plasma level of dopa increased significantly and it accumulated in the striatum and hypothalamus even though the dose of L-dopa (10 mg kg^{-1}) given with benserazide was much lower than that used alone (50 mg kg^{-1}). Also the plasma levels of DOPAC and HVA were significantly reduced suggesting that, despite the increased level of dopa, less dopa was metabolised to dopamine whilst more was excreted unchanged (Table 1).

With chronic dosing the advantages of adding benserazide were less marked. Although it gave a significantly higher plasma level of dopa (5.67 ± 0.85), than L-dopa alone (0.09 ± 0.06 μM) this was not reflected by increased striatal levels of dopa (9.25 ± 1.50 μM without and 9.42 ± 3.33 μM with benserazide). This may be a consequence of the very high plasma levels of 3-MT modifying dopa penetration into the brain (see below) but, nevertheless, the combined treatment still achieved significantly higher dopamine levels than L-dopa alone (56.5 ± 2.63, cf. 34.9 ± 2.45 μM). However, since this latter figure also showed a significant increase over control values (25.5 ± 0.90 μM), it would be important to know to what extent the extra dopamine achieved with benserazide can be used. One might conclude that if it accumulates it is not used. Certainly, although the small elevation of striatal dopamine seen after chronic L-dopa (25.5 to 34.9 pmol mg^{-1}) alone is accompanied by increased production of its metabolites DOPAC and HVA, the much larger increase in dopamine (to 56.3 pmol g^{-1}) after L-dopa and benserazide shows no further increase in the metabolites, although urine HVA is increased, and some of this may come from the brain. Thus, the higher levels of dopamine found in the benserazide animals may not be followed by its increased release and turnover.

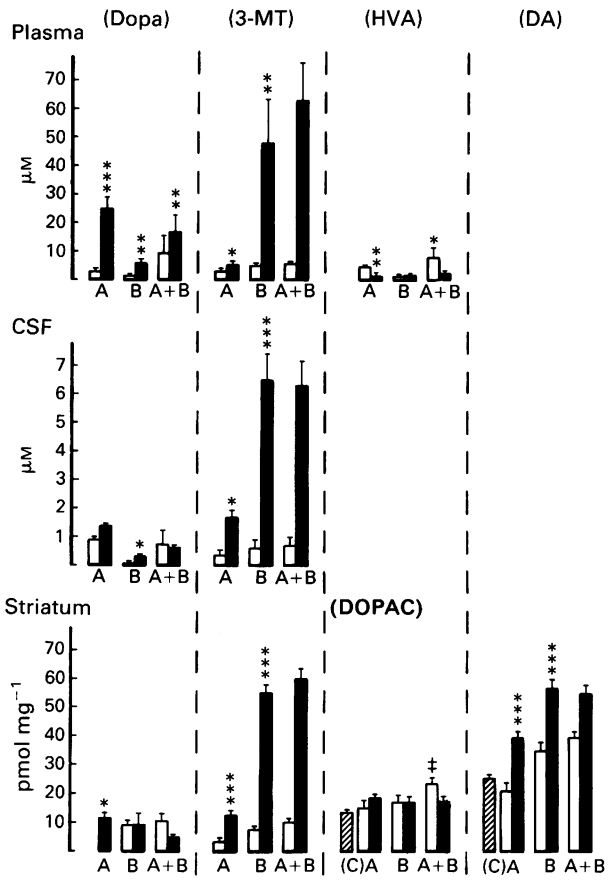

Figure 1 Comparison of the levels of dopa, 3-methoxytyrosine (3-MT) and dopamine (DA) in the plasma, CSF and striatum of rats and either 3,4-dihydroxyphenylacetic acid (DOPAC) (striatum) or homovanillic acid (HVA) (plasma) after either L-dopa alone (open columns) or L-dopa with benserazide (solid columns) given either acutely (A) or chronically (B). The levels in rats treated chronically but also subsequently challenged with an acute administration of L-dopa alone (open columns) or with benserazide (solid columns) are shown as A + B. For doses see Methods. None of the substances was detected in the plasma and CSF of untreated animals but dopamine, DOPAC and HVA were present in the striatum and their levels are shown as controls (C). Each column represents the mean value and bars show s.e., $n = 6$. Significant effects produced by the addition of benserazide compared to L-dopa alone are shown as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ whilst † ($P < 0.05$) shows a significant effect of an acute challenge on chronically treated animals (A + B).

The most striking effect of benserazide, either acutely or chronically, was the significant increase in 3-MT in plasma, CSF and brain, especially in the striatum where it reached a concentration comparable with that of dopamine. It is most probable that 3-MT is formed peripherally and transported into the brain because; (1) its concentration in the hypothalamus of chronically-treated animals was similar to that in the striatum (Table 1) despite the much lower level of dopa (and dopamine) in the hypothalamus. (2) Since 3-MT formation is favoured by blocking the decarboxylation of dopa, it should not be produced very readily in the CSF and brain, to which the decarboxylase inhibitor does not penetrate. Certainly 3-MT blocks the transport of systemically administered [^{14}C]-dopa into rat brain but not peripheral tissues (Gervas *et al.*, 1983). It also stopped the restoration of brain dopamine levels from systemically administered dopa in animals pretreated with α-methyltyrosine.

If 3-MT competes with L-dopa for uptake into the brain its high concentration and long half-life could have considerable consequences on the effects of further dosing with L-dopa. Thus, although chronic administration of L-dopa alone to rats for six weeks had little influence on the distribution and metabolism of a subsequent single injection (Table 3, Figure 1), greater increases in striatal dopamine, DOPAC and HVA were seen than when L-dopa was given to naïve animals. The same was not true for animals treated chronically with L-dopa plus benserazide in which the levels of 3-MT were much higher. In this group the subsequent administration of the same drug combination produced smaller increases in striatal dopa, dopamine, 3-MT, DOPAC and HVA, as well as plasma dopa, than its acute injection into naïve animals (Table 3). Of course the level of dopamine in the striatum could have been so high after chronic treatment with L-dopa plus benserazide (56.3 pmol mg^{-1}) compared with normal animals (25.5), that no more could be formed and (or) stored. Alternatively, additional dopamine synthesis might have been impaired by the low levels of dopa in the striatum of these animals. Again this raises the question of whether the high levels of 3-MT in all compartments of animals treated chronically, especially if given benserazide as well as L-dopa, affect not only the absorption of any subsequently administered dopa but also its metabolism to dopamine. Melamed *et al.* (1983) also found that if rats were dosed with L-dopa (100 mg kg^{-1} day^{-1} i.p) and carbidopa (25) for 30 days, then subsequent challenge with the same drug combination produced significantly smaller increases in striatal dopamine than when untreated animals were given the same challenge. Surprisingly, their chronically-treated animals did not have raised striatal dopamine levels before challenge. Also, since they used a different ExCDDI in a smaller dose relative to L-dopa and did not measure 3-MT the results are not strictly comparable.

From our studies it appears that when L-dopa is given chronically alone in appropriate doses it is as effective in producing increased levels of striatal dopa as when given the benserazide and, although lower levels of dopamine are achieved (34.9 compared to 56.3 pmol mg^{-1}) these could be adequate. Certainly these results indicate that the administration of benserazide with L-dopa either acutely or chronically can produce such high concentrations of 3-MT, in the brain, that a full analysis of the effects of 3-MT on dopamine function is required. Thus, although L-dopa needs to be given in much higher doses alone than when used with benserazide, the persisting high concentrations of 3-MT might impair its effectiveness and even contribute to its slowly developing failure in Parkinsonism. Admittedly the ratio of benserazide to dopa was much higher in these studies than that used clinically and could produce a disproportionate increase in 3-MT. Nevertheless, whilst little is known about the brain levels of 3-MT in man it is found in high concentrations in plasma. Mena *et al.* (1986) showed that patients with a high plasma 3-MT: dopa ratio suffered a significantly higher incidence of 'on-off' fluctuations and Feuerstein *et al.* (1977) found a significantly higher level of plasma 3-MT in patients with dyskinesias than in those without. By contrast Fahn (1974) and Luguin *et al.* (1989) could find no correlation between changes in circulating 3-MT and motor performance. Also, if 3-MT is reducing dopa penetration into the brain it is surprising that it is associated with increased dyskinesia which relies on an overactive dopaminergic system.

Our chronic studies also show that adequate brain levels of dopa and dopamine can be obtained with quite low plasma levels provided the drug is administered in a continuing manner. We added L-dopa to the drinking water and, although we did not monitor drinking patterns, it is unlikely that the input was as constant as that achieved with miniature pumps in man. Trials with this approach, however, show that whilst swings in motor performance are reduced they are not abolished by infusions of L-dopa and ExCDDIs (Hardie *et al.*, 1984; 1986; Nutt *et al.*, 1984).

In transposing data from experimental studies of this kind to the clinical situation, it should be remembered that the pattern of metabolism of levodopa in the central nervous system of Parkinsonian patients may be different from that seen in normal rats with a complete nigrostriatal pathway. In fact, since 3-MT is formed peripherally and can cross the

blood brain barrier its effects could be even more important in patients with reduced striatal dopamine function.

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