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Re-evaluation of the L-dopa loading effect on dopamine metabolism in rat striatum

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Investigation by HPLC with electrochemical detection of dopamine (DA) metabolism in rat striatum after L-dopa + benserazide treatment allowed quantification of the time course evolution of DA, 3,4-dihydroxyphenylacetic acid and homovanillic acid levels. Furthermore, four peaks which did not appear in controls, were detected in treated striatum. One was identified as 3-methoxytyrosine, the level of which was still high 9 h after treatment. 3-Methoxytyrosine, has been detected previously in plasma of parkinsonian patients treated with L-dopa, and the disturbance in DA metabolism could explain some of the side-effects induced by that treatment.

L-Dopa with benserazide has been widely used in the treatment of Parkinson's disease (Bartholini et al 1968). L-Dopa loading has been examined in rats for its effects on conditioned avoidance response (Seiden & Martin 1971), circling behaviour (Melamed et al 1984) and 'bizarre social behaviour' (Lammers & van Rossum 1968).

The evaluation of the central biochemical effects of this treatment up to date has been essentially with fluorimetric methods. However, these did not permit the detection of unexpected metabolic modifications. Since 1980, methods that associate a high performance liquid chromatographic separation with an electrochemical detection (LC-ECD) have become available. The detection limit, the specificity, and the wide range of analytical possibilities of these LC-ECD methods allow the simultaneous determination of a large number of catechols and indoles in the same sample. On the chromatograms, in addition to the known peaks, other compounds can be co-eluted and investigated. With these methods, several authors have studied the L-dopa loading effect on the dopamine (DA) metabolism in various tissues (Hefti et al 1981; Beers et al 1984; Ehrenstrom & Johansson 1985).

By using this method, we aimed to investigate the effects of L-dopa + benserazide treatment, and focused our attention on the levels of DA and its metabolites in rat striatum.

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Materials and methods

Male, Sprague Dawley rats (Charles River, France), 225–250 g, maintained under standard conditions were used. The schedule of the treatment was according to a 'balanced lattice design' (Cochran & Cox 1957). Animals were injected with L-dopa (125 mg kg^{-1} i.p.), 30 min after pretreatment with benserazide hydrochloride (50 mg kg^{-1} i.p.). Treatment began every day at 0930h. Control rats received i.p. 0·9% NaCl (saline) injections. Decapitation was 0·5, 1, 1·5, 3, 4·5, 6, 7·5 and 9 h after L-dopa administration. The brain was rapidly removed and striatum isolated on a plate chilled to −15 °C. The samples were stored at −80 °C until assayed. Each time, 5 treated and 5 control rats were used, to take into account eventual circadian changes on the levels of DA and its metabolites. Sample preparation and chromatographic procedure were according to a LC-ECD method previously described (Orosco et al 1985). All chemicals used were of nanograde purity. L-Dopa and benserazide were generously supplied by Roche laboratories; catechol compounds used in the external standard mixture (Fig. 1A) were from Sigma.

Results

The qualitative and quantitative study took into consideration the dopaminergic compounds. In Fig. 1(B), the chromatogram from a control striatum showed the dopaminergic peaks usually measured (3,4-dihydroxyphenylacetic acid (DOPAC). DA and homovanillic acid (HVA). In Fig. 1(C), the chromatogram from a treated rat exhibited the same dopaminergic peaks plus four unexpected peaks. One of these has been identified as 3-methoxytyrosine (3-*O*-Me-dopa), which in the control striatum was barely detectable. In order to verify the identity of this peak, retention times of 3-*O*-Me-dopa

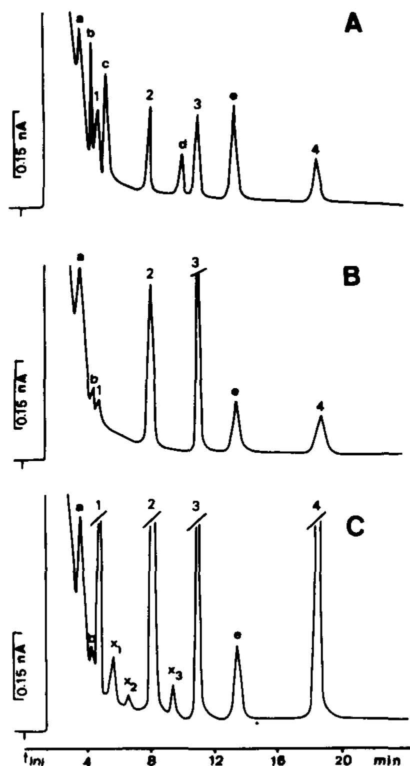

FIG. 1. Experimental chromatograms from a standard mixture (0·5 ng/injection of each compound) (A), striatum of an untreated rat (B) and striatum of a rat killed 1·5 h after treatment with L-dopa + benserazide (C). Chromatographic conditions: mobile phase = K-phosphate 0·1 M + EDTA 0·1 mM + heptane sulphonic acid 5 mM (Pic B7 Waters) + methanol 7·5%. pH = 3·85. Column: reversed phase silica gel (Ultrasphere ODS 5 μm, L = 15 cm, id = 4·5 mm Beckman). Detection: potential = 0·8 V vs Ag/AgCl reference electrode. Sensitivity 1 nA full scale deflection. Numbers in chromatograms indicate: 3-*O*-Me-dopa (1), 3,4-dihydroxyphenylacetic acid (DOPAC) (2), dopamine (3) and homovanillic acid (HVA) (4). Because of the large amount of (1), (2), (3) and (4) in treated striatum, the measurements were performed on diluted samples. a, b, c, d and e were 3-methoxy-4-hydroxyphenylethylene glycol, noradrenaline, adrenaline, metanephrine and 5-hydroxyindoleacetic acid and were not studied in this work.

have been evaluated for various pH values of the mobile phase. The retention times were similar in biological samples and standard solutions.

All the dopaminergic derivatives increased in concentration (Table 1). The evolution of their levels, versus time, showed a rapid augmentation, noticeable after 30 min (at least 150%). The highest increase was observed 1 h after administration for DA (+310%), 3 h for DOPAC (+2650%) and 4·5 h for HVA (+1630%). The maximal concentration of 3-*O*-Me-dopa occurred 6 h after L-dopa treatment (27247 ± 4197 ng g^{-1}). The DA baseline was restored 6 h after DA administration. For DOPAC and HVA, the times were 7·5 and 9 h, respectively. In contrast, even after 9 h, the 3-*O*-Me-dopa concentration remained high and accurately measurable.

Discussion

After L-dopa + benserazide treatment, two phenomena were observed: (1) a large increase in DA metabolism and (2) the presence of several unknown peaks on the chromatograms. To our knowledge, the time course of formation and degradation of DA metabolites after L-dopa + benserazide treatment in rat striatum has not been studied in this way before.

Ehrenstrom & Johansson (1985), using a similar LC-ECD method, noticed in the striatum of L-dopa-treated rats that the highest concentration of DA (+137%) was obtained after 30 min and the maximal increase in DOPAC levels (+370%) occurred after 60 min. They also investigated three other DA metabolites (salsolinol, norsalsolinol and epinine) and reported the presence of an unknown peak on the chromatograms from treated rats.

Our results show that benserazide enhances the exogenous L-dopa effect by increasing its central bioavailability, leading to a larger and longer lasting effect on DA metabolism. As previously shown by Ho & Smith (1983) in rat hypothalamus, benserazide alone (50 mg kg^{-1} i.p.) does not modify DA levels. Moreover, by using our LC-ECD method we verified that in the striatum of rats treated by benserazide alone, none of the four unknown peaks was detectable.

Among the four unusual peaks observed after L-dopa treatment one was identified as 3-*O*-Me-dopa. This was previously detectable by LC-ECD in the serum of L-dopa treated patients with Parkinsonism (Beers et al 1984). It is likely that the other three peaks also have dopamine-related structures.

As our chromatographic conditions were close to those of Ehrenstrom & Johansson, it may be assumed that the unknown peak described by those authors is 3-*O*-Me-dopa.

Kuruma et al (1970) reported a considerable accumulation of [^{14}C]3-*O*-Me-dopa in rat brain after repeated administration of [^{14}C]dopa. Furthermore, it has been suggested that plasma levels and 3-*O*-Me-dopa/L-dopa ratio may be predictive indicators of the long term response to L-dopa therapy (Beers et al 1984).

The very high increase in DA, DOPAC and HVA levels and the appearance of non-classical metabolites in the central nervous system after treatment with L-dopa + benserazide, could explain, at least partially, the various side effects and the general decline in efficiency observed in clinical experience, in the treatment of Parkinson's disease by this drug combination.

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Table 1. Effect of L-dopa + benserazide treatment on DA, DOPAC, HVA and 3-*O*-Me-dopa levels in rat striatum.

Time (h)	DA		DOPAC		HVA		3-*O*-Me-dopa	
	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.
0·5	7630 ±938	*** 28 018 ±2496	1592 ±222	*** 10 704 ±1399	868 ±77	*** 2 223 ±213	ND	4 332 ±1238
1·0	7266 ±760	*** 29 788 ±3108	1358 ±214	*** 20 986 ±1486	767 ±96	*** 4 166 ±220	ND	8 603 ±843
1·5	8994 ±1593	*** 26 939 ±3543	1331 ±108	*** 29 727 ±7916	727 ±90	*** 6 378 ±883	ND	11 646 ±2692
3·0	8297 ±308	** 17 797 ±1650	1383 ±119	*** 38 021 ±8525	682 ±93	*** 11 208 ±1980	ND	23 202 ±6794
4·5	8565 ±809	* 10 176 ±997	1224 ±145	*** 15 344 ±3365	652 ±97	*** 11 313 ±147	ND	23 525 ±4623
6·0	8818 ±895	8 327 ±625	1356 ±277	* 3 321 ±1 450	840 ±92	*** 9 681 ±963	ND	27 247 ±4197
7·5	8700 ±1027	7 676 ±989	1268 ±166	1 343 ±147	540 ±52	** 2 869 ±1449	ND	23 381 ±8588
9·0	8579 ±1013	7930 ±893	1124 ±49	1 152 ±217	648 ±70	665 ±266	ND	15 436 ±8384

Values are mean ± standard deviation for n = 5 determinations, expressed in ng g^{-1} of striatum.
Cont. = control striatum, Treat. = treated striatum, ND = not detectable.
$^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ between control and treated.

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