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METABOLISM OF CARBIDOPA TO ALPHA-METHYLDOPAMINE AND ALPHA-METHYLNOREPINEPHRINE IN RATS

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Abstract—Recent observations on the central and peripheral actions of carbidopa (CD) combined with our own results with the compound led us to examine its metabolism and effects on brain catecholamines in rats. CD was found to undergo a two-stage N-deamination process *in vivo* giving rise to alpha-methyldopa (AMD) and alpha-methyldopamine respectively. Further, beta-hydroxylation yielded alpha-methylnorepinephrine. These metabolic products were demonstrated in rat brain with reductions in norepinephrine and 3-methoxy-4-hydroxyphenylglycol, and little effect on dopamine. These results are consistent with the alpha-2 agonist effects of alpha-methylnorepinephrine. The relative formation of alpha-methyldopamine from CD was about 26% of an equivalent dose of AMD. It is concluded that some of the central effects of CD may be mediated by its metabolism to AMD, which readily crosses the blood–brain barrier. Possible implications of the findings are discussed.

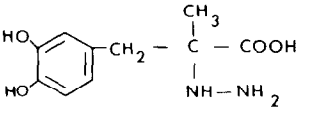
Carbidopa (CD) (alpha-methyldopa hydrazine) is widely used in combination with levodopa in the treatment of Parkinson's disease. CD inhibits *l*-aromatic amino acid (dopa) decarboxylase in the peripheral system (since it does not cross the blood–brain barrier). Theoretically, it reduces the peripheral catabolism of levodopa, increasing its availability for entrance into the brain [1–3]. Recent studies indicate that CD raises hypothalamic dopa and serum prolactin in rats [3], increases serum prolactin (but not growth hormone or cortisol) secretion in man [4, 5], inhibits norepinephrine (NE) formation in sympathetic nerves [6], and has an additive anti-hypertensive effect in combination with a wide range of medications including hydrochlorothiazide [7]. These effects have been explained by the inhibition of dopa decarboxylase but may be explainable by a different mechanism.

In evaluating the actions of the monoamine oxidase-β inhibitor (-)-deprenyl, we studied its effects in combination with the levodopa-carbidopa compound (Sinemet), in Parkinsonian patients [8]. Unexpectedly, we observed the excretion of large amounts of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid. It was speculated that this was a result of incomplete inhibition of dopa decarboxylase by CD. In subsequent work (F. Karoum *et al.*, unpublished), we studied the effects of CD on the steady-state excretion of several biogenic amines and found little influence on catecholamines and serotonin, but marked reduction in the formation of phenylethylamine and tyramine. This seemed to indicate different effects on subspecies of *l*-aromatic amino acid decarboxylase and led us to consider alternative mechanisms of action for CD.

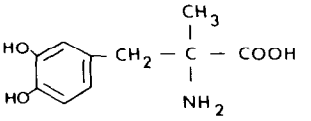
The main metabolic pathway described previously for the degradation of CD involves the removal of the N-hydrazine group from the compound, with various metabolites formed thereafter [9]; however, careful analysis of excretion products failed to demonstrate the hydrazine group. In observing the similarities of CD to alpha-methyldopa (AMD) (Fig. 1), we postulated a two-stage N-deamination with significant formation of AMD as an intermediary step. We designed an experiment to test this hypothesis which may have implications in the treatment of certain movement disorders.

MATERIALS AND METHODS

CD and AMD were obtained from Merck, Sharp & Dohme (West Point, PA). Deuterated DA ($^2H_4$-DA) and NE ($^2H_3$-NE) were purchased from Merck & Co. Inc. (St. Louis, MO). Alpha-methyldopamine



Carbidopa



Alphamethyldopa

Fig. 1. Structures of carbidopa and alpha-methyldopa.

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Table 1. Brain dopamine (DA), norepinephrine (NE), and 3-methoxy-4-hydroxyphenylglycol (MHPG), and formation of alpha-methyldopamine (AMDA) and alpha-methylnorepinephrine (AMNE) after administration of carbidopa and alpha-methyldopa*

	Hypothalamus					Septal nuclei				Caudate-putamen			
	DA	NE	MHPG	AMDA	AMNE	DA	NE	AMDA	AMNE	DA	NE	AMDA	AMNE
Controls (saline)	4.00 ± 0.84	30.7 ± 1.0	1.88 ± 0.12			9.94 ± 1.20	8.45 ± 0.72			117.2 ± 6.1	1.11 ± 0.17		
Carbidopa,† 25 mg/kg	5.08 ± 0.61	24.5 ± 1.0‡	1.78 ± 0.06			10.14 ± 0.75	7.61 ± 0.53			112.1 ± 7.5	0.8 ± 0.11		
Carbidopa, 50 mg/kg	3.9 ± 0.43	19.4 ± 2.9‡	1.74 ± 0.21	0.094 ± 0.031	11.5 ± 5.35	14.76 ± 2.14§	7.12 ± 1.34	0.1 ± 0.0	2.39 ± 0.41	115.1 ± 11.0	1.19 ± 0.24	0.37 ± 0.25	0.22 ± 0.03
Alpha-methyldopa, 25 mg/kg	4.22 ± 0.50	6.7 ± 1.1‡	1.17 ± 0.18	0.38 ± 0.14	19.83 ± 0.94	11.69 ± 1.95	2.40 ± 0.24‡	0.23 ± 0.08	4.38 ± 0.39	94.3 ± 8.3§	0.79 ± 0.08	0.36 ± 0.05	0.36 ± 0.04
Alpha-methyldopa, 50 mg/kg	4.44 ± 0.8	5.0 ± 0.50‡	0.98 ± 0.11‡	0.77 ± 0.14	19.48 ± 0.97	11.85 ± 3.18	1.76 ± 0.18‡	0.59 ± 0.10	4.54 ± 0.39	99.6 ± 2.7	0.69 ± 0.068	0.85 ± 0.11	0.40 ± 0.05

* Results are expressed as mean ± S.E.M. in ng/mg protein.
† Rats were administered the carbidopa and alpha-methyldopa intragastrically twice per day for 4 days and the morning of day 5; decapitation was performed 90 min after the last dose (five rats per group).
‡ $P < 0.005$, two-tailed t-test.
§ $P < 0.05$, two-tailed t-test.
‖ $P < 0.02$, two-tailed t-test.

and alpha-methylnorepinephrine were obtained from the Regis Chemical Co. (Morton Grove, IL). All other chemicals were of the highest quality commercially available and were obtained from the Fischer Scientific Co. (Pittsburgh, PA).

Male Sprague–Dawley rats (150–200 g, Zivic-Miller, Allison Park, PA) were distributed into five groups of five rats each: (1) CD 25 mg/kg, (2) CD 50 mg/kg, (3) AMD 25 mg/kg, (4) AMD 50 mg/kg, and (5) normal saline controls. All animals in the drug-treatment groups were administered medication twice per day intragastrically for 4 days and the morning of day 5. Ninety minutes after the last dose all the animals were decapitated, and the hypothalamus, caudate-putamen, and septal nuclei were dissected according to Glowinski and Iversen [10] and placed on dry ice until analyzed (within 1 hr).

Brain samples were homogenized in 0.5 ml 1 N HCl containing 50 ng of $^2H_3$-NE and $^2H_4$-DA and centrifuged at 12,000 g for 5 min; the clear supernatant fraction was transferred to another tube and stored at −16°. Total protein was determined from 10 μl of the tissue homogenate before centrifugation by the method of Lowry *et al.* [11]. NE, DA and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured by mass fragmentography as previously reported [12]. Alpha-methyldopamine and alpha-methylnorepinephrine were measured simultaneously with DA and NE by focusing on fragments m/z 442 and 604 respectively.

A Finnigan model 4000 gas-chromatograph quadrupole mass-spectrometer was employed. All derivatives and gas-chromatograph parameters were as previously reported [12].

RESULTS

The results of the analyses are summarized in Table 1. All brain areas demonstrated formation of alpha-methyldopamine and alpha-methylnorepinephrine with CD and AMD. Comparing the concentrations of alpha-methyldopamine in the hypothalamus after 25 mg/kg of AMD versus 50 mg/kg of CD yielded a ratio of about 4:1. Assuming that the concentration of brain alpha-methyldopamine is linearly related to the doses of AMD and CD administered, it can be calculated that about one-fourth of the 25 mg/kg AMD (6.25 mg/kg) will produce the same hypothalamic concentration of alpha-methyldopamine as did 50 mg/kg of CD. Thus, from considering the hypothalamus, it seems that about 12% of the CD ingested by the rats is metabolized to alpha-methyldopamine. Employing a similar argument for the septal nuclei and caudate-putamen, percentages of 19 and 47% can be derived respectively. Combining the percentages of alpha-methyldopamine found in the three brain areas yields an overall mean of 26%. Therefore, between 10 and 30% of the CD ingested by the rat is apparently deaminated to AMD.

Each area demonstrated formation of alpha-methyldopamine from AMD (25 and 50 mg/kg) in the expected amounts relative to dose (i.e. roughly 2:1); however, all brain areas showed approximately equal formation of alpha-methylnorepinephrine from AMD 25 and 50 mg/kg.

Norepinephrine was reduced significantly ($P < 0.005$) from control values in the hypothalamus with CD and AMD. The reduction in MHPG achieved statistical significance with AMD (25 and 50 mg/kg), but it was not lowered to the same degree as NE. Significant reduction of NE in septal nuclei and caudate-putamen also occurred with AMD.

There was little effect on DA throughout, with actually an *increase* (achieving significance at the 0.05 level) noted in the septal nuclei with CD 50 mg/kg. There was also a trend toward increase of DA in the hypothalamus with the lower dose of CD, but it did not reach statistical significance. Additionally, there was a slight lowering of DA in the caudate-putamen with AMD 25 mg/kg. The mechanisms responsible for lowering the brain NE content do not seem to operate the same upon brain DA.

DISCUSSION

Levodopa therapy of Parkinson's disease represented a major advance over previous medical and surgical treatments [13]. However, side effects of the medication including nausea, vomiting, cardiac arrhythmias, and hypotension limited its usefulness. The combination of putative dopa decarboxylase inhibitors [14, 15] with levodopa allowed significant reduction of the dose, thereby ameliorating the substantial side effects [16–19]. CD is now accepted as a safe and effective compound in enhancing the central effect of levodopa, and the combination has gained nearly universal acceptance in the treatment of Parkinson. CD is believed to inhibit peripheral decarboxylation of *l*-dopa enabling more to enter the brain. In view of our finding, another mechanism may also come into play.

In our experience, we could not demonstrate decreases in excreted DA and its metabolites until an intragastric dose of 500 mg/kg of CD was achieved (F. Karoum, unpublished). Much previous research on the compound used either *in vitro* enzymatic assays [1, 20] or higher dose intraperitoneal injections [3, 6]. Since the dose of CD in man is approximately 1–2 mg/kg (in combination with four to ten times that of levodopa), we tested metabolism at relatively lower doses of CD in the rat (25 and 50 mg/kg). Our results show the formation of alpha-methyldopamine and alpha-methylnorepinephrine. About 26% of the ingested CD was calculated to be converted to AMD. In this context it should be noted that, at the relatively modest doses employed here, both CD and AMD showed preferential effects on brain NE and its metabolism when compared to DA. Thus, as shown in Table 1, a substantial reduction of NE was noted, especially in the hypothalamus with some concomitant decrease in MHPG. There was little effect, however, on DA. The reduction of NE by CD and AMD may be the result of competitions from alpha-methyldopamine for dopamine beta-hydroxylase in the storage granules. Since DA concentrations were not affected proportionately as that of NE, it may be that the enzyme systems controlling the synthesis of DA (including dopa decarboxylase) were not markedly affected by the amounts of CD and AMD employed. AMD likely has several actions in the CNS, but its main function is through its metabolism to alpha-methylnorepinephrine, a potent alpha-2 receptor agonist. We posit that our results may be consistent with this model.

What then is the effect of AMD in Parkinson's disease? At relatively higher doses (greater than 500 mg/day), the medicine has been known to induce a Parkinsonian-like syndrome with bradykinesia, psychomotor retardation, rigidity, and tremor, which has been attributed to relative DA depletion due to the formation of alpha-methyldopamine [21–23]. AMD has been shown, however, to reverse reserpine-induced bradykinesia and tremor [24–27]; this effect is blocked by dopa decarboxylase inhibitors (such as RO 4-4602), dopamine-beta-hydroxylase inhibitors (such as FLA-63 and disulfiram), and noradrenergic blockers (for example, phenoxybenzamine), but not by the dopamine-specific blocker pimozide. These results indicate that the ability of AMD to reverse the reserpine-induced bradykinesia and tremor is mediated via a noradrenergic mechanism. AMD has been demonstrated to improve some patients with Parkinsonism, particularly when used at lower doses, but this has not been consistently replicated [28–31]. If CD is metabolized to alpha-methyldopamine and alpha-methylnorepinephrine at a rate of 25% of AMD, only an amount equal to about 20–30 mg/day of AMD would be formed. At low doses, we have demonstrated little effect on DA formation, and a relatively more substantial change in NE; we have shown both reduced accumulation of NE (possibly by either competition with dopamine beta hydroxylase or competition for uptake by alpha-methylnorepinephrine) and its turnover, as reflected in reduced MHPG, consistent with the model of reserpine-reversal mediated by NE reduction described above. What remains to be demonstrated are the specific noradrenergic mechanisms in movement disorders such as Parkinsonism.

Dopamine activity is modulated by NE innervation in the amacrine cells of the retina; here, tyrosine hydroxylase (the rate-limiting enzyme in the formation of DA) shows reduced activity (and therefore less DA formation) with alpha-2 adrenergic receptor blockade, which increases NE [32]. The reciprocal relationship between NE and DA (as well as other transmitters such as serotonin, gamma-aminobutyric acid, etc.) is seen in several areas, such as the regulation of TSH secretion, which is stimulated by NE and diminished by DA activity [33–37]. Antelman and Caggiula [38] have proposed a model of dynamic interactions for the DA and NE systems; in this scheme, at a baseline level of functioning, NE exerts an indirect modulatory effect on DA. Here, the functional activity of the DA system is inversely correlated with NE. They propose that, when one of the systems is diminished, the other compensates by increasing its activity when the organism is under stress. Further, they state that in situations where one side is diminished in function, this might be corrected by either increasing the activity of the previously diminished transmitter or decreasing the other. In Parkinson's disease, this would involve augmenting DA or reducing NE activity. Our data indicate that, at relatively low doses, the AMD for-

med from CD may have the effect of inhibiting NE availability, which may produce some beneficial effects and which may explain some of the more recent findings with CD described previously. One interesting possibility is that certain symptom complexes (such as bradykinesia or tremor) may be dependent upon alterations in different neurotransmitters, and that these may be modified independently.

We have demonstrated the formation of alpha-methyldopamine and alpha-methylnorepinephrine from CD in rats with concomitant reduction NE. Whether this occurs in man, and whether it has any functional significance (i.e. improving or exacerbating Parkinsonian symptoms) is unknown. However, it does raise the possibility of noradrenergic effects in the use of CD in Parkinsonism. Further work is being done comparing the effects of CD on the excretion of DA metabolites in animals.

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REFERENCES

1. C. C. Porter, L. S. Watson, D. C. Titus and J. A. Totaro, *Biochem. Pharmac.* **11**, 1067 (1962).
2. J. R. Bianchine, in *The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), p. 475. Macmillan, New York (1980).
3. C. J. Watkins, J. G. Wiggins and R. J. Wurtman, *Life Sci.* **24**, 1675 (1979).
4. G. M. Brown, P. E. Garfinkel, J. J. Warsh and H. C. Stancer, *J. clin. Endocr. Metab.* **43**, 236 (1976).
5. P. E. Garfinkel, J. J. Warsh, H. C. Stancer, D. D. Godse, G. M. Brown and M. Vranic, *Neurology* **27**, 443 (1977).
6. R. J. Wurtman and C. J. Watkins, *Nature, Lond.* **265**, 79 (1977).
7. A. Scriabine, C. T. Ludden, C. A. Stone, R. J. Wurtman and C. J. Watkins, *Clin. Sci. molec. Med.* **51**, 407s (1976).
8. F. Karoum, L-W. Chuang, T. Eisler, D. B. Calne, M. R. Liebowitz, F. M. Quitkin, D. F. Klein and R. J. Wyatt, *Neurology* **32**, 503 (1982).
9. S. Vickers, E. K. Stuart, J. R. Bianchine, H. B. Hucker, M. E. Jaffe, R. E. Rhodes and W. J. A. VandenHeuvel, *Drug Metab. Dispos.* **2**, 9 (1974).
10. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. F. Karoum, in *Methods in Biogenic Amine Research* (Eds. S. Parvez, T. Nagatsu, I. Nagatsu and H. Parvez), p. 237. Elsevier, Amsterdam (1983).
13. G. C. Cotzias, P. S. Papavasiliou and R. Gellene, *New Engl. J. Med.* **280**, 337 (1974).
14. W. G. Clark, *Pharmac. Rev.* **11**, 330 (1959).
15. V. J. Lotti and C. C. Porter, *J. Pharmac. exp. Ther.* **172**, 406 (1970).
16. H. Mars, *Archs Neurol. Chicago* **28**, 91 (1973).
17. D. B. Calne, J. L. Reid, S. D. Vakil, S. Rao, A. Petrie, C. A. Pallis, J. Gawler, P. K. Thomas and A. Hilson, *Br. med. J.* **3**, 729 (1971).
18. C. D. Marsden, P. E. Barry, J. D. Parkes and K. J. Zilkha, *J. Neurol. Neurosurg. Psychiat.* **36**, 10 (1973).
19. C. H. Markham, S. G. Diamond and L. J. Treciokas, *Archs Neurol., Chicago* **31**, 128 (1974).
20. L. Airoldi, C. J. Watkins, J. F. Wiggins and R. J. Wurtman, *Metabolism* **27**, 771 (1978).
21. B. M. Groden, *Br. med. J.* **1**, 1001 (1963).
22. R. A. Vaidya, A. B. Vaidya, M. H. Van Woert and N. G. Kase, *Metabolism* **19**, 1068 (1970).
23. M. J. T. Peaston, *Br. med. J.* **2**, 168 (1964).
24. J. Necina, *Life Sci.* **7**, 301 (1962).
25. N. J. Uretsky and L. S. Seiden, *J. Pharmac. exp. Ther.* **168**, 153 (1969).
26. R. C. Duvoisin and C. Marsden, *Brain Res.* **71**, 178 (1974).
27. R. C. Duvoisin and C. D. Marsden, in *Advances in Neurology* (Eds. D. B. Calne, T. N. Chase and A. Barbeau), p. 243. Raven Press, New York (1975).
28. R. J. Mones, *NY St. J. Med.* **1**, 47 (1974).
29. D. O. Marsh, H. Schneiden and J. Marshall, *J. Neurol. Neurosurg. Psychiat.* **26**, 505 (1963).
30. R. D. Sweet, J. E. Lee and F. H. McDowell, *Clin. Pharmac. Ther.* **13**, (1972).
31. J. Fermaglich and D. S. O'Doherty, *Trans. Am. neurol. Ass.* **96**, 231 (1971).
32. P. M. Iuvone and A. L. Rauch, *Life Sci.* **33**, 2455 (1983).
33. S. Zgliczynski and M. Kaniewski, *Acta endocr., Copenh.* **95**, 172 (1978).
34. P. T. Mannisto, J. Mattila and J. Tuomisto, *Acta endocr., Copenh.* **97**, 213 (1981).
35. G. Delitala, L. Devilla, A. Canessa and F. D'Asta, *Acta endocr., Copenh.* **98**, 521 (1981).
36. T. R. Hall and J. Meites, *Gen. Pharmac.* **12**, 485 (1981).
37. P. Mannisto, J. Mattila and S. Kaakkola, *Eur. J. Pharmac.* **76**, 403 (1981).
38. S. M. Antelman and A. R. Caggiula, *Science* **195**, 646 (1977).