CARBIDOPA ELEVATES HYPOTHALAMIC DOPA AND SERUM PROLACTIN IN RATS¹

Carol J. Watkins, Jane F. Wiggins and Richard J. Wurtman

Laboratory of Neuroendocrine Regulation
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

(Received in final form March 19, 1979)

Summary

The administration of carbidopa (MK-486, α-methyl-L-dopa hydrazine; 100-200 mg/kg, intraperitoneally) causes several-fold increases in hypothalamic dopa and serum prolactin levels in male rats within 2 hours; these changes are not reversed by the administration of pyridoxine. These observations suggest that high doses of carbidopa can affect catecholamine synthesis within the hypothalamus.

Carbidopa, an inhibitor of aromatic L-amino acid decarboxylase (AAAD) in peripheral tissues, has been shown to suppress catecholamine synthesis in cardiac sympathetic nerves (1). A number of observations previously have been interpreted as showing that carbidopa is unable to cross the blood-brain barrier. For example, doses as high as 1000 mg/kg reportedly caused no detectable changes in brain decarboxylation in vivo (2); the administration of ¹⁴C-labelled carbidopa (5-20 mg/kg, to dogs) yielded no measurable levels of radioactivity in cerebral tissues (3,4).

Recent studies have shown, however, that the administration of high doses of carbidopa can elevate serum prolactin (PRL) levels in humans (5) and in rats (6,7). The tuberoinfundibular dopaminergic tract located within the hypothalamus apparently is critical in inhibiting the release of PRL from the anterior pituitary. Since the median eminence of the hypothalamus lacks a true anatomic blood-brain barrier (8), neurons in this region conceivably could be perfused with carbidopa when the drug is given systemically.

Carbidopa administration causes a reduction in hypothalamic AAAD activity, as assayed in vitro (7,9); this reduction can be reversed (9) by the administration of pyridoxine (PYR), which is the precursor for pyridoxal-5'-phosphate (PLP), the cofactor for

¹These studies were supported by grants from the Barra Foundation and the United States Public Health Service (AM-14223). Jane F. Wiggins was the recipient of National Institutes of Health Fellowship 1F32 CA05297-01 from the National Cancer Institute.
decarboxylation. The present studies were undertaken to determine whether the effects of carbidopa on PRL release were modified by the administration of PYR and whether these effects could be correlated with changes in hypothalamic decarboxylase activity in vivo. Using a sensitive isotopic assay (10) to measure endogenous levels of dopa, the substrate for AAAD in catecholaminergic neurons, we found that high doses of carbidopa do affect AAAD within the hypothalamus, that the changes roughly parallel the PRL response, and that neither change is reversed by PYR administration.

Materials and Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 150-350 g were housed in suspended metal cages. They were exposed to light (Vita-Lite, Duro-Test, North Bergen, N.J.) daily from 8 AM to 8 PM and had free access to food (Charles River Rat Chow) and tap water.

Carbidopa (generously provided by Merck, Sharp & Dohme Co., West Point, Pa.) was injected intraperitoneally (i.p.) as a suspension in water or saline. Control rats received water or saline (0.9%) in equal volumes. The hydrochloride salts of 5-hydroxybenzylhydrazine (NSD-1015; Aldrich, Milwaukee, Wis.) and pyridoxine (Sigma Chemical Co., St. Louis, Mo.) were dissolved in water and neutralized with NaOH to pH 7.0-7.6 before i.p. injection.

Except as noted below, rats were killed by decapitation. Blood was collected from the cervical wound and stored briefly on ice, then centrifuged for immediate assay of serum dopa or storage at -20°C for later assay of serum PRL. Brains were removed immediately and dissected on an ice-cold plate; brain regions were frozen on dry ice until they were homogenized. The median eminence-basal hypothalamus and pituitary stalk (referred to collectively as ME) were dissected in some studies; in most experiments, however, the entire hypothalamus was used.

For the isotopic dopa microassay, hypothalami were homogenized in 250-500 μl of 0.1 M HClO4, ME regions in 125 μl, and slices of frontal cortex weighing 50-100 mg in 500 μl; 100 μl of serum was mixed with 150-400 μl of the acid. The homogenates were allowed to stand in ice for 30 minutes before being centrifuged at 3000 g for 10 minutes. Aliquots of 40 μl, with or without internal standards of 1 ng dopa (Regis, Morton Grove, Ill.), were assayed by incubation with catechol-O-methyl-transferase (COMT) and 3H-(methyl)-S-adenosyl-methionine (3H-SAM) at 37°C for 90 minutes, as described by Hefti and Lichtensteiger (10). In our assay, each tube contained 0.8 x 10^-4 μmole of 3H-SAM (New England Nuclear, Boston, Mass.; 11 mCi/μmole), 1.2 x 10^-4 μmole of SAM-iodide (Sigma), 0.075 μmole of MgCl2·6H2O (Fisher Scientific, Fair Lawn, N.J.), 50 μg of dithiothreitol (Cleland's reagent, J.T. Baker, Phillipsburg, N.J.), and 25 μl of COMT (prepared from rat liver according to the procedure of Coyle and Henry [11], up to the dialysis step; here aliquots were frozen at -20°C, in a total volume of 200 μl of 0.28 M Tris-acetate buffer (Sigma Trizma) at pH 8.6. The labelled 3-O-methyl dopa was isolated by adsorption on Dowex cation and anion exchange resins (Bio-Rad, Richmond, Calif.) and charcoal. The standard curve was linear for quantities of dopa from 0.1 to 5 ng. Carbidopa does not interfere with this assay in amounts likely to be present within the hypothalamus (< 5 ng).
Decapitation of rats reportedly can cause a post-mortem decrease in brain dopa content (measured fluorimetrically), relative to death by microwave irradiation (12). To eliminate the possibility that the experimentally-induced changes in hypothalamic dopa were artifacts of the killing procedure, we carried out one experiment in which animals were sacrificed by 3-second exposure to a 2450 MHz, 1116 Watt beam focused on the head in a modified Litton microwave oven. The rats were positioned upside-down in the holder to ensure complete inactivation of hypothalamic enzymes. Using the isotopic dopa assay, we found that both control and carbidopa-treated rats sacrificed by irradiation showed hypothalamic dopa levels comparable to those observed in tissues from animals killed by decapitation. (Data obtained in other experiments suggest that whole brains and pineals of control rats killed by irradiation have higher dopa levels than those from rats killed by decapitation).

Serum PRL was determined by radioimmunoassay (RIA) using materials provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases; rat PRL RP-1 served as the reference preparation. Results are reported as means \( \pm \) SEM. Data were analyzed using the Student's t-test or, for more than two groups, one-way analysis of variance (ANOVA) and Dunnett's test for multiple comparisons with a single control (13).

Results

In four separate experiments, endogenous dopa levels in the hypothalamus increased after acute administration of carbidopa (100 mg/kg, 2 hours); they rose from 0.031 \( \pm \) 0.004 \( \mu \)g/g for controls to 0.097 \( \pm \) 0.012 \( \mu \)g/g for drug-treated rats \((p < 0.001)\). These data support the idea that carbidopa can inhibit dopa decarboxylase in at least part of the hypothalamus. However, carbidopa was clearly less active than NSD-1015 (an inhibitor known to cross the blood-brain barrier) which, in an equal dose (100 mg/kg), elevated hypothalamic dopa levels tenfold, to 0.319 \( \pm \) 0.021 \( \mu \)g/g within one hour \((p < 0.001)\) compared with controls or with carbidopa-treated rats.

The carbidopa-induced increase in hypothalamic dopa apparently was dose-related (Table 1) and linear with time (Fig. 1). Significant changes were observed only at a high dose (100 mg/kg) and after 2 hours. These conditions were used in all subsequent experiments, except as noted below.

Since the median eminence is known to contain dopaminergic neurons involved in the control of PRL release, we examined carbidopa's effect on dopa levels in the ME. Dopa was significantly elevated in rats receiving the drug \((0.116 \pm 0.013 \text{ ng/ME} [p < 0.01],\) compared to \(0.048 \pm 0.009 \text{ ng/ME for control rats}\). It should be noted that the dopa content of the ME in control rats yielded counts of \(^3\)H-3-O-methyl dopa that were only 20% above blank values.

In order to determine whether the apparent increases in the dopa contents of the ME and hypothalamus might simply be caused by the presence of dopa in the blood perfusing these tissues, we measured serum dopa levels in rats similarly treated. In two separate experiments, serum dopa levels rose about fourfold after carbidopa treatment \((12.3 \pm 2.0 \text{ ng/ml} [p < 0.01],\) compared with \(3.0 \pm 0.4 \text{ ng/ml in control animals}\). Inasmuch as circulating blood represents only a small percentage of the mass of brain tissue, and
since blood dopa levels were lower than those of the brain tissues (25-50 ng/g) in both groups of animals, a carbidopa-induced rise in blood dopa cannot account for the apparent increases in hypothalamic or ME dopa.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hypothalamic Dopa (µg/g)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>0.022 ± 0.003</td>
</tr>
<tr>
<td>Carbidopa 10 mg/kg</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>Carbidopa 30 mg/kg</td>
<td>0.030 ± 0.002</td>
</tr>
<tr>
<td>Carbidopa 100 mg/kg</td>
<td>*0.058 ± 0.010</td>
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</tbody>
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Groups of 4 or 5 rats were injected with 0.9% saline or carbidopa (10, 30, or 100 mg/kg; i.p.) 2 hours before decapitation. The correlation coefficient for the dose/response line is 0.9914. Data are expressed as means ± SEM.

* *p < 0.005 differs from controls (ANOVA, Dunnett's test)

Dopa levels in small sections of frontal cortex of rats receiving carbidopa failed to increase significantly. Hence it seems unlikely that higher centers are responsible for carbidopa's effects on the hypothalamus.

We previously demonstrated that carbidopa administration lowered hypothalamic PLP levels, while its co-administration with PYR maintained normal tissue PLP concentrations and eliminated the drug-induced decrease in AAAD activity assayed in vitro (9). In contrast, PYR administration (200 mg/kg, 30 minutes before the AAAD inhibitor) failed to block the carbidopa-induced rise in hypothalamic dopa levels; both groups of rats had high dopa levels (0.145 ± 0.018 µg/g in rats given supplemental PYR and 0.141 ± 0.022 µg/g in animals receiving only the carbidopa).

Serum PRL was elevated after acute administration of carbidopa to male rats; this response was also dose-related, and attained significance (p < 0.05) 2 hours after rats received 200 mg/kg (69.5 ± 24.5 ng/ml compared with 14.4 ± 4.9 ng/ml for controls). Rats killed 2 hours after receiving the last of 7 injections of carbidopa (100 mg/kg given twice a day) also exhibited a consistent elevation in serum PRL (188.0 ± 39.0 ng/ml compared with 42.0 ± 6.0 ng/ml in control animals [p < 0.01]). Repeated lower doses (5 or 10 mg/kg on the same injection schedule) failed to affect serum PRL. In rats given a series of 3 carbidopa injections (100 mg/kg each, at 8-hour intervals), serum PRL was elevated 2 hours after the final dose regardless of whether PYR was also given (Table 2).
Discussion

These data demonstrate that, in high doses, (a) carbidopa can inhibit the decarboxylation of endogenous dopa in the hypothalamus, resulting in an accumulation of dopa, and (b) the drug can significantly elevate serum PRL levels; neither of these effects is blocked by the co-administration of PYR. Our data do not show that these effects occur at carbidopa doses (5-10 mg/kg) more comparable to those used clinically.

TABLE 2

Effect of Co-administration of PYR and Carbidopa on Serum PRL Levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>19.3 ± 3.1</td>
</tr>
<tr>
<td>Carbidopa</td>
<td>*105.7 ± 20.6</td>
</tr>
<tr>
<td>PYR</td>
<td>16.9 ± 1.4</td>
</tr>
<tr>
<td>Carbidopa + PYR</td>
<td>*103.7 ± 16.2</td>
</tr>
</tbody>
</table>

Groups of 7 rats received 3 pairs of injections over a period of 24 hours. On each occasion, vehicle or PYR (200 mg/kg) was given first and was followed 20 minutes later by either vehicle or carbidopa (100 mg/kg). Animals were decapitated 2 hours after the final injection. Data are expressed as means ± SEM. *p < 0.01 differs from controls (ANOVA, Dunnett's test)

The increase in hypothalamic dopa implies that at least part of this region is accessible to carbidopa, and supports earlier observations that carbidopa given in vivo reduces hypothalamic AAAD activity as assayed in vitro (7,9). These data also confirm the inability of PYR to fully block the inhibition of AAAD by carbidopa: PYR had no effect on the carbidopa-induced administration of dopa in the hypothalamus, just as it had earlier been shown not to block carbidopa's inhibition of in vivo decarboxylation of exogenous dopa by the heart and kidney (9). Taken together, these observations indicate that carbidopa does not inhibit AAAD simply by reducing the available level of its cofactor. (It should be noted that our previous experiments [9] did suggest that carbidopa's inhibition of hypothalamic AAAD activity could be reversed with PYR. However, that assay for AAAD was an in vitro method, which required homogenization of the tissues, and, consequently, the mixing of drug, cofactor, enzyme, and other tissue constituents. Thus, it may not have reflected in vivo drug effects on AAAD as dependably as dopa accumulation does.)

The carbidopa-induced suppression of hypothalamic catecholamine synthesis may be related to the concurrent increase in serum PRL. Extensive evidence is available implicating the tuberoinfundibular dopaminergic tract in inhibiting the release of PRL from the anterior pituitary. Drugs that block dopamine (D2) receptors (e.g.,
pimozide) or interfere with DA synthesis (α-methyltyrosine) increase circulating PRL levels (14,15). On the other hand, drugs that mimic DA (e.g., apomorphine) or those that stimulate DA synthesis (e.g., L-dopa), reduce serum PRL (16,17). It is not known whether the DA released from the tuberoinfundibular neurons acts locally to stimulate secretion of a PRL-release-inhibiting factor (PIF) from the hypothalamus (18), or whether it is transported via the pituitary-portal circulation to act directly on the pituitary (19). The observations that DA is present in pituitary-portal blood (20), that there are DA receptors on the pituitary but apparently not on the basal hypothalamus (21), and that DA inhibits the release of PRL from the pituitary in vitro (22) all support the latter hypothesis.

FIGURE 1

Time Course for Hypothalamic Dopa Accumulation after Carbidopa Administration

Groups of 6-12 rats were killed 2 hours after receiving saline, or one or 2 hours after receiving carbidopa (100 mg/kg, i.p.). The correlation coefficient of the line is 0.9999.

†p < 0.005 differs from controls (ANOVA, Dunnett’s test)

The PRL regulatory system may have characteristics which make it especially sensitive to small changes in DA synthesis. Tuberoinfundibular neurons, unlike other dopaminergic neurons, are resistant to the actions of 6-hydroxy-dopamine (23) and d-amphetamine (24); this suggests that they may lack a mechanism for taking up, and possibly reutilizing, previously released DA. Furthermore, neurotransmitter release may preferentially involve newly-formed catecholamines (25,26). These factors might amplify the effects of a reduction in DA synthesis.

Our data cannot rule out the possibility that carbidopa might act directly on the pituitary to stimulate PRL synthesis and/or release. In vitro experiments have shown that drugs such as DA receptor blockers (e.g., haloperidol, phenothiazines) and TRH, a small peptide, can act in this way (27). However, α-methyltyrosine, the drug most closely related in structure to carbidopa among those that have been tested, had no direct effect on pituitary PRL release (J. F. Wiggins and J. D. Fernstrom, submitted for publication).
Since carbidopa is an inhibitor of AAAD, other brain neurons utilizing monoaminergic transmitters—for example, noradrenergic or serotonergic neurons—could be affected by its administration and might account for its effect on PRL. Norepinephrine's role in the control of PRL secretion is unclear; however, serotonin-containing neurons can, under certain conditions, stimulate PRL release (27). Thus, if carbidopa acted by inhibiting hypothalamic serotonin synthesis, it would be expected to inhibit PRL release. Dopaminergic neurons are the most likely site of carbidopa's effect on PRL release; this is supported by abundant evidence that DA inhibits PRL secretion and that brain regions, such as the median eminence, which lie within the brain but are not protected by an anatomically-defined blood-brain barrier, are rich in DA terminals.

The present observations indicate that the administration of very high doses of carbidopa can reduce dopamine synthesis in the tuberoinfundibular neurons and thereby increase serum PRL levels in male rats. Since lower doses (5-10 mg/kg) had no effect on hypothalamic dopa or serum PRL levels in the animals, our observations are not in themselves any basis for concern about similar responses in humans.

References