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Enhancement of the anti-hypertensive effect of methyldopa and other anti-hypertensive drugs by carbidopa in spontaneously hypertensive rats

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Summary

1. A peripheral inhibitor of L-aromatic amino acid decarboxylase, carbidopa [(−)-L-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid monohydrate], at doses up to 25 mg/kg intraperitoneally or 30 mg/kg orally had no effect on directly recorded arterial pressure of spontaneously hypertensive rats derived from the Wistar/Okamoto strain. It enhanced, however, the anti-hypertensive effects of methyldopa, hydrallazine, guanethidine and clonidine, and, to a lesser extent, reserpine and hydrochlorothiazide. The mechanism of this enhancement is presently unknown, but biochemical studies support the assumption that carbidopa is likely to reduce sympathetic nervous system activity.

2. The conversion of [^{3}H]tyrosine (given intraperitoneally) to dopa (3,4-dihydroxyphenylalanine) and catecholamines was measured in the hearts and adrenals of control rats and animals pretreated with carbidopa (100 mg/kg, intraperitoneally). Carbidopa significantly decreased the accumulation of ^{3}H-labelled catecholamines in both organs and increased their total tyrosine content and the specific radioactivity of tyrosine.

Key words: anti-hypertensive drugs, carbidopa, drug interaction, methyldopa.

Introduction

Carbidopa is a peripheral inhibitor of L-aromatic amino acid decarboxylase and does not cross the blood–brain barrier (Lotti & Porter, 1970; Clark, Oldendorf & Dewhurst, 1973). The anti-hypertensive effect of methyldopa is attributed to the central effect of its metabolite, α-methylnoradrenaline (Henning & van Zwieten, 1968; Torchiana, Lotti, Clark & Stone, 1973). A peripheral inhibitor of decarboxylase can be expected to inhibit peripheral metabolism of methyldopa and increase its concentration in various tissues including brain. In species like rats, where decarboxylation of methyldopa is appreciable (Porter & Titus, 1963), the inhibition of the peripheral decarboxylation by carbidopa can lead to increased formation of α-methylnoradrenaline in the central nervous system and, hence, increased anti-hypertensive activity. In addition, peripheral vasoconstrictor action of α-methylnoradrenaline cannot be expected to oppose its central anti-hypertensive effect. We have demonstrated the enhancement of the anti-hypertensive action of methyldopa by carbidopa in spontaneously hypertensive rats. In these animals, carbidopa enhanced the activity of all other anti-hypertensive drugs tested by us.

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Methods

Spontaneously hypertensive rats of the Wistar/Okamoto strain were purchased from Charles River/Lakeview Co. (Wilmington, Mass., U.S.A.). Arterial pressure was recorded in conscious male rats of 250–350 g body weight by a direct technique involving cannulation of the caudal artery (Watson, 1976). The pressure was recorded continuously through Statham P23Gb transducers on a Honeywell 906C Visicorder. Mean arterial pressure and heart rate data were printed at 30 min intervals through a data acquisition system (Data Graphics Corp., San

Antonio, Texas, U.S.A.) by means of ASR-33 teletype units. All drugs were administered in volumes of 2 ml/kg. Carbidopa, methyldopa and clonidine were dissolved in acidified (HCl) distilled water and further diluted to required concentrations; guanethidine and hydrallazine were dissolved in distilled water; reserpine was dissolved in acetic acid/ethanol (1:5) (14 ml of mixture/g of reserpine) and further diluted with distilled water; hydrochlorothiazide was dissolved in alkaline (NaOH) distilled water and further diluted to the required concentration. The doses of all drugs were expressed in terms of base weight. In experiments involving intraperitoneal administration of two drugs, carbidopa was given 5 min before the anti-hypertensive drugs; in experiments involving oral treatment, carbidopa was given 30 min before methyldopa.

The anti-hypertensive drugs were tested intraperitoneally at three to six doses each, alone and with carbidopa. Three to nine rats were used at each dose of each drug. Methyldopa was tested at 2·5, 10 and 40 mg/kg. The following drugs were tested at six doses each, ranging from 0·03 to 2·0 mg/kg for hydrallazine, from 0·75 to 200 μg/kg for clonidine and from 0·078 to 5·0 mg/kg for guanethidine. Reserpine was tested at 0·6, 2·5 and 10 μg/kg and hydrochlorothiazide at 5, 20, 40 and 80 mg/kg. In experiments involving methyldopa intraperitoneally with or without carbidopa, an incomplete 8×6 randomized block design was used (Cochran & Cox, 1957). Eight treatments involved three doses of methyldopa with and without carbidopa, carbidopa alone and acidified sodium chloride solution (150 mmol/l), 2 ml/kg. Six rats were used in each treatment group. In experiments involving oral treatment, methyldopa was given at 5, 20 or 40 mg/kg 30 min after carbidopa (30 mg/kg). Calculations of the relative potency and 95% confidence limits were based on procedures described by Finney (1964).

In studies involving evaluation of the effects of carbidopa on noradrenaline synthesis, a total of forty male Sprague–Dawley rats of 150–175 g body weight were injected with a suspension of carbidopa (100 mg/kg) intraperitoneally, or its diluent (50 mmol/l HCl). One hour later, 100 μCi of 3,5-L-[^{3}H]tyrosine (specific radioactivity $9{\cdot}78 \times 10^{10}$) was injected intraperitoneally. The total volume injected per rat was less than 1 ml. After 1 h the rats were decapitated and the heart and adrenals removed and frozen. The tissues were homogenized in perchloric acid (0·4 mol/l) containing ethylenediaminetetraacetic acid (EDTA) (200 μl of a 10% solution) and sodium metabisulphite (50–100 μl of a 5% solution). The supernatants were separated on Dowex and alumina columns; the resulting tyrosine, dopa and catecholamine fractions were counted in a Packard Tri-Carb 3330 scintillation spectrometer. The total tyrosine was determined from the fluorescence of the 1-nitroso-2-naphthol derivative, according to a modified method of Waalkes & Udenfriend (1957), and measured on an Aminco–Bowman spectrophotofluorimeter.

Results

Carbidopa, 25 mg/kg intraperitoneally, had no effect on the arterial pressure alone but significantly enhanced the anti-hypertensive effects of methyldopa, hydrallazine, clonidine, guanethidine, reserpine and hydrochlorothiazide. The average control mean arterial pressure values in spontaneously hypertensive rats varied from 165 to 185 mmHg. The anti-hypertensive effect of drugs was dose-dependent and, at doses used, ranged from a 10 to a 50 mmHg fall in arterial pressure. The relative potencies of anti-hypertensive drugs with and without carbidopa were determined on the basis of the maximal effects over a 24 h period (Table 1). Potencies of all anti-hypertensive drugs tested were significantly higher in rats treated with carbidopa than in untreated animals. Carbidopa shifted the dose–response regression lines of the anti-hypertensive drugs to the left with little or no change in the maximal obtainable anti-hypertensive effect or in the duration of action. The minimal dose of carbidopa required for the enhancement of the anti-hypertensive effect of

TABLE 1. *Effect of carbidopa on relative potency of various anti-hypertensive drugs in spontaneously hypertensive rats*

Drug	Relative potency with carbidopa (25 mg/kg i.p.)[1]	95% confidence limits
Methyldopa	3·6	1·8–9·9
Hydrallazine	5·6	2·1–23·2
Guanethidine	3·6	1·9–8·4
Reserpine	2·4	1·0–5·9
Clonidine	5·5	2·5–18·0
Hydrochlorothiazide	1·7	1·1–2·8

[1] Potency of each anti-hypertensive drug alone = 1.

hydrallazine was estimated to be 1·25 mg/kg intraperitoneally; with other anti-hypertensive drugs, carbidopa was not tested at doses lower than 25 mg/kg intraperitoneally. Carbidopa and methyldopa were also given orally to thirteen spontaneously hypertensive rats. Carbidopa, 30 mg/kg orally, plus methyldopa, 40 mg/kg orally 30 min later, had, at 4 h after treatment, a greater anti-hypertensive effect than methyldopa alone at the same dose (35 vs. 17 mmHg fall in arterial pressure).

Hydrallazine-induced cardiac acceleration was not significantly modified by carbidopa, whereas clonidine-induced bradycardia was enhanced by carbidopa. Clonidine (50 μg/kg intraperitoneally) alone, slowed the heart rate at 30 min after treatment by an average of 77 beats/min; the corresponding value in carbidopa-pretreated animals was 139 beats/min.

In normotensive rats treated with [^{3}H]tyrosine, carbidopa (100 mg/kg intraperitoneally) elevated cardiac tyrosine from 13·1 to 28·9 μg/g of tissue (average values for twenty rats, $P < 0.001$) and increased specific radioactivity of cardiac tyrosine from 7·2 to $12{\cdot}7 \times 10^5$ d.p.m./μmol ($P < 0.001$) and of adrenal tyrosine from 11·3 to $17{\cdot}6 \times 10^5$ d.p.m./μmol ($P < 0.01$). Cardiac and adrenal ^{3}H-labelled catecholamines (corrected for specific radioactivity of tyrosine) were decreased by carbidopa from 11·5 to $4{\cdot}7 \times 10^{-3}$ and from 4·4 to $3{\cdot}0 \times 10^{-2}$ μmol/g of tissue respectively. These decreases were significant statistically ($P < 0.001$ for heart and $P < 0.02$ for adrenals). Carbidopa also significantly decreased the accumulation of ^{3}H-labelled catecholamines and increased the accumulation of ^{3}H-labelled DOPA in hearts of spontaneously hypertensive rats.

Discussion

The anti-hypertensive drugs used in this study, except methyldopa, are not substrates for amino acid decarboxylase. The enhancement of their effects by carbidopa cannot therefore be explained by an increase in the concentration of their metabolites in the central nervous system. An impairment of catecholamine synthesis in peripheral sympathetic neurons by carbidopa can explain the observed enhancement of the anti-hypertensive drugs. Our findings suggest that, at sufficiently high doses, carbidopa, by virtue of its peripheral decarboxylase inhibitory activity, can reduce the synthesis of noradrenaline. This, alone, may not be sufficient to reduce arterial pressure but may conceivably enhance the activity of drugs known to interfere with sympathetic transmission by another mechanism (clonidine, reserpine, guanethidine). With methyldopa, an increase in α-methylnoradrenaline in the central nervous system and a reduction of its formation in peripheral tissues are likely to play an additional role in the enhancement of the anti-hypertensive action by carbidopa in the rat. It should be pointed out that decarboxylation is a more important metabolic reaction for methyldopa in the rat (Porter & Titus, 1963) than it is in man (Buhs, Beck, Speth, Smith, Trenner, Cannon & Laragh, 1964). It is therefore conceivable that, in man, carbidopa will not enhance the anti-hypertensive activity of methyldopa to the same extent as in the rat.

Peripheral vasodilator drugs, e.g. hydrallazine, are likely to increase sympathetic tone by a reflex effect initiated by vasodilatation and lowering of arterial pressure. This reflex vasoconstriction and cardiac acceleration usually oppose vasodilatation in the maintenance of arterial pressure. Inhibition of peripheral decarboxylase by carbidopa may interfere with this regulatory mechanism even though hydrallazine-induced cardiac acceleration was, in our experiments, not significantly affected by carbidopa. Similar interference with the regulatory mechanisms may explain the observed slight enhancement of the anti-hypertensive action of hydrochlorothiazide by carbidopa.

References

Buhs, R.P., Beck, J.L., Speth, O.C., Smith, J.L., Trenner, N.R., Cannon, P.J. & Laragh, J.H. (1964) The metabolism of methyldopa in hypertensive human subjects. *Journal of Pharmacology and Experimental Therapeutics*, **143**, 205–214.

Clark, W.G., Oldendorf, W.H. & Dewhurst, W.G. (1973) Blood–brain barrier to carbidopa (MK-486) and Ro 4-4602 peripheral dopa decarboxylase inhibitors. *Journal of Pharmacy and Pharmacology*, **25**, 416–418.

Cochran, W.G. & Cox, G.M. (1957) *Experimental Design*, pp. 95–153. John Wiley, New York.

Finney, D.J. (1964) *Statistical Methods in Biological Assay*, 2nd edn, pp. 99–227. Hafner Publishing Co., New York.

Henning, M. & van Zweiten, P.A. (1968) Central hypotensive effect of α-methyldopa. *Journal of Pharmacy and Pharmacology*, **20**, 409–417.

Lotti, V.J. & Porter, C.C. (1970) Potentiation and inhibition of some central actions of L(−)-DOPA by decarboxylase inhibitors. *Journal of Pharmacology and Experimental Therapeutics*, **172**, 406–415.

Porter, C.C. & Titus, D.C. (1963) Distribution and metabolism of methyldopa in the rat. *Journal of Pharmacology and Experimental Therapeutics*, **139**, 77–87.

Torchiana, M.L., Lotti, V.J., Clark, C.M. & Stone, C.A. (1973) Comparison of centrally mediated hypotensive

action of methyldopa and DOPA in cats. *Archives Internationale de Pharmacodynamie et de Therapie*, **205**, 103–113.

WAALKES, T.P. & UNDENFRIEND, S. (1957) A fluorometric method for the estimation of tyrosine in plasma and tissues. *Journal of Laboratory and Clinical Medicine*, **50**, 733–736.

WATSON, L.S. & LUDDEN, C.T. (1976) Continuous recording of arterial blood pressure in conscious rats. In: *New Antihypertensive Drugs*. Ed. Scriabine, A. & Sweet, C.S. Spectrum Publications, Holliswood, N.Y. (In press).