These findings suggest that imipramine may impair the hypotensive action of clonidine by increasing the responsiveness of noradrenaline at the neuroeffector cells of the cardiovascular system. Since arterial hypertension is associated with hyper-reactivity of blood vessels to vasoconstrictor agents (Somlyo & Somlyo, 1970; Haeusler & Finch, 1972), and imipramine potentiates the peripheral action of catecholamines (Osborne & Sigg, 1960), the effect of noradrenaline and angiotensin II on vascular reactivity in the perfused hindquarter preparation was studied for this reason in control and SH rats treated for 8 days.

Noradrenaline (0·5 μg, i.a.) and angiotensin II (0·5 μg, i.a.) elicited the pressor responses in perfusion pressure. However, the same doses of these agents failed to show any appreciable effect in systemic blood pressure. Pretreatment with clonidine (0·2 mg kg^{-1}, orally for 8 days) did not alter the responses to noradrenaline and angiotensin II, whereas imipramine (2·0 mg kg^{-1}, orally for 8 days) significantly potentiated ($P < 0.01$) the responses to these agents. Imipramine + clonidine pretreatment showed increased ($P < 0.01$) vasoconstrictor responses to noradrenaline and angiotensin II compared with that of control and clonidine-treated groups (Fig. 3).

From these studies it appears that pretreatment with imipramine would build up a high concentration of catecholamines at the peripheral cells by inhibition of the neuronal uptake. This action of imipramine may counteract the decrease in peripheral sympathetic activity induced by clonidine thereby antagonizing its hypotensive action.

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Effects of neutral amino acids on the antihypertensive action of methyldopa in spontaneously hypertensive rats

F. G. ZAVISCA, R. J. WURTMAN*, *Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.*

The mechanism by which methyldopa (α-methyl-3,4-dihydroxyphenylalanine) lowers blood pressure probably involves its uptake into the central nervous system (cns) and its decarboxylation to form an amine (Henning & Van Zweiten, 1968; Chalmers & Wurtman, 1971; Heise & Kroneberg, 1973; Van Zweiten, 1973; Maitre, Hedwall & Waldmeier, 1974; Chalmers, 1975; Scriabine, Clineschmidt & Sweet, 1976a; Scriabine, Ludden & others, 1976b). Inhibition of the enzyme aromatic amino acid decarboxylase (AAAD) within the cns thus abolishes methyldopa's antihypertensive effect, while inhibition of AAAD only in peripheral organs not only fails to block the fall in blood pressure, but actually potentiates it (Henning & Van Zwieten, 1968; Scriabine & others, 1976b). That a cns locus mediates methyldopa's antihypertensive action is also suggested by the observation that application of the methyldopa metabolite α-methylnoradrenaline to various brainstem and hypothalamic loci also lowers blood pressure (DeJong, Nijkamp & Bohus, 1975; Struyker-Boudier, Smeets & others, 1975; DeJong & Nijkamp, 1976).

Since methyldopa is a large neutral amino acid (LNAA) similar in structure to the naturally occurring amino acids phenylalanine and tyrosine, one might expect that its transport from the blood to the brain would be mediated by the same specific uptake system as that transporting the LNAAs normally present in

* Correspondence.

blood (Pardridge & Oldendorf, 1975; 1977; Wurtman & Fernstrom, 1976). Indeed, competition among methyldopa and other amino acids for transport in the kidney and intestine is well documented (Young & Edwards, 1964; 1966), and recent studies from our institution have shown that a concurrent injection of LNAAs can decrease the proportion of a methyldopa dose that is subsequently detected in the brain of the normal rat (Markovitz & Fernstrom, 1977). The following observations provide evidence that plasma concentrations of LNAAs can also determine the magnitude of methyldopa's effects on blood pressure.

Male spontaneously hypertensive rats of the Okamoto strain (Charles River Laboratories, Wilmington, Mass.), 230–320 g, having systolic blood pressures of 180–220 mm Hg, were exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, N.J.) from 07.00 to 13.00 h daily and given free access to food and water (Charles River Rat-Mouse-Hamster formula, 24% protein). Food was removed at noon on the day before an experiment, and all experiments were begun soon after the onset of the daily light period (07.00 h). Systolic blood pressure was measured by the tail cuff method (see Udenfriend, 1976) using a Narco Biosystems Pneumatic Pulse Transducer. Rats were warmed at 38° for 15 min before each reading, and at least six readings were obtained and averaged for each time point. Rats were pre-conditioned by measuring blood pressure on at least four occasions on as many days. At least six days were allowed to pass between successive experiments using a given group of rats.

In each experiment, blood pressure was measured immediately before an injection and again 3 h later (the interval after which, in preliminary studies, LNAAs maximally inhibited methyldopa's actions). Rats were maintained at ambient temperatures of 28° and given free access to water during the 3 h interval. Methyldopa (3, 10, 25, 50, 100, or 250 mg kg^{-1}), alone or in combination with LNAAs (the mixture or phenylalanine alone), was suspended in 0·05 N HCl and injected intraperitoneally with a 20-gauge needle. In one series of experiments, an LNAA mixture was used (25 mg kg^{-1} each of phenylalanine, tyrosine, leucine, isoleucine, and valine, making 125 mg kg^{-1} total, or 0·88 mmol kg^{-1}). In the second series, phenylalanine was administered in a concentration equimolar to the above mixture (146 mg kg^{-1}). Preliminary experiments showed that the amino acid mixture had no effect on blood pressure.

In one experiment, rats were given methyldopa, 100 mg kg^{-1}, alone or with the LNAA mixture, and were decapitated immediately after the second determination of blood pressure. Methyldopa concentrations in the whole spinal cord were measured fluorometrically (Dominic & Moore, 1971).

Nine or 10 animals were used in each group. Results were expressed as change in blood pressure, in mm Hg, for each rat (in relation to its own control values); data from each group were averaged, and statistical significance between groups was assessed by Student's *t*-test.

The concurrent administration of the LNAA mixture or of phenylalanine produced a highly significant attenuation of methyldopa's effect on blood pressure in the middle dose range (25–100 mg kg^{-1}) (Figs 1 and 2). The effect of the LNAAs on blood pressure among rats receiving 100 mg kg^{-1} of methyldopa (i.e. to reduce it to 37·7 ± 3·7 mm Hg; as opposed to 58·0 ± 7·2 mm Hg in animals receiving only the methyldopa; $P < 0.002$) paralleled the decrease in spinal cord methyldopa concentrations (6·5 ± 0·4 μg kg^{-1} in rats receiving LNAAs plus methyldopa *vs* 10·5 ± 0·06 μg g^{-1} in animals receiving only methyldopa, $P < 0.001$).

The effect of methyldopa was dose-related up to the point of maximum response (i.e. about 100 mg kg^{-1}); the highest dose used (250 mg kg^{-1}) failed to decrease blood pressure any further, but did override the suppressive effect of the LNAA mixture and of phenylalanine (Figs 1 and 2).

These observations suggest that, by competing with methyldopa for uptake into the cns, LNAAs decrease the proportion of each dose reaching the proposed site of action where it can be converted to biologically active metabolites that act on monoaminergic receptors and thereby attenuate the resulting fall in blood pressure.

Phenylalanine was tested alone because it has the lowest Km for the brain LNAA transport system (Pardridge & Oldendorf, 1975). It was thus expected to bind more tightly to the LNAA carrier (at the

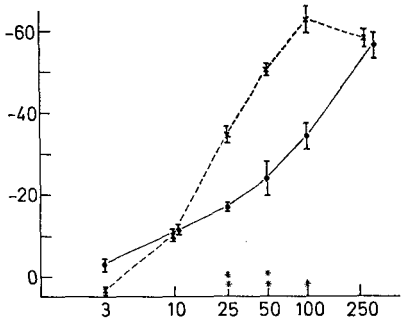

FIG. 1. Relation between dose of methyldopa, with or without concurrent administration of LNAAs, and fall in systolic blood pressure among spontaneously hypertensive rats. Each point represents the mean ± s.e.m. of the fall in blood pressure (in mm Hg) 3 h after rats received methyldopa alone (×) or methyldopa plus large neutral amino acids (●) intraperitoneally. Each point represents the mean obtained from 9 or 10 rats. $*P < 0.01$ differs from rats receiving methyldopa alone. $**P < 0.001$ differs from rats receiving methyldopa alone. Ordinate: Change in blood pressure (mm Hg). Abscissa: α-Methyldopa (mg kg^{-1}).

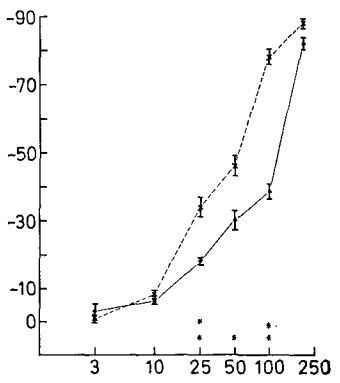

FIG. 2. Relation between methyldopa dose, with or without concurrent administration of phenylalanine, and fall in systolic blood pressure among spontaneously hypertensive rats. Each point represents the mean ± s.e.m. of the fall in blood pressure (in mm Hg) 3 h after rats received methyldopa alone (×) or methyldopa plus phenylalanine (●) intraperitoneally. Each point represents the mean obtained from 9 or 10 rats. $*P < 0{\cdot}01$ differs from rats receiving methyldopa alone. $**P < 0{\cdot}001$ differs from rats receiving methyldopa alone. Ordinate: Change in blood pressure (mm Hg). Abscissa: α-Methyldopa (mg kg^{-1}).

blood-brain barrier) than the other LNAAs and, hopefully, to cause a greater inhibition of methyldopa uptake than the LNAA mixture. This was not observed (Figs 1 and 2), possibly because phenylalanine was more rapidly metabolized than some of the other LNAAs. Hence, the concentrations of LNAAs acting at the blood-brain barrier might not have been comparable. No other single amino acid has been tested.

The exact loci at which methyldopa metabolites act to lower blood pressure are currently unknown. They might involve the brainstem, the synapses made by descending bulbospinal neurons (Chalmers & Wurtman, 1971; Scriabine & others, 1976a) or other sites in the cns. LNAAs could be expected to reduce methyldopa uptake at any of these loci. None of the LNAAs that we administered is thought to have any significant direct effect (at the doses used) on the enzymes involved in methyldopa's biotransformation once the drug is taken up into the cns. It seems likely that the amino acid pattern of the plasma will be found to be a determinant of the effectiveness not only of methyldopa, but also of any cns-active drug that happens to be a neutral amino acid (Ordonez, Ambrus & others, 1974).

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