Arterial Blood Pressure in Dogs: Effects of *m*-Tyrosine Alone or in Combination with Inhibitors of Aromatic Amino Acid Decarboxylase; Relative Hypotensive Potencies of L-Dopa, DL-*m*-Tyrosine, and L-*m*-Tyrosine

We reported that injection of L-dopa produces a centrally-mediated reduction in arterial blood pressure in dogs if they are first pretreated with Ro 4-4602 (N^1-DL-seryl-N^2-2, 3, 4-trihydroxybenzylhydrazine) or carbidopa (L-HMD)[1]. Carbidopa is the L-isomer of DL-α-methyl-α-hydrazino-3, 4-dihydroxyphenylpropionic acid (HMD). The L-form has been shown to be the active component of the racemic mixture[2]. ROBSON[3] reported similar results with L-dopa methyl ester in dogs which were likewise pretreated with inhibitors of aromatic amino acid decarboxylase. A hypotensive response was observed when rats received HMD plus L-dopa[4,5]. Metabolites of L-dopa were thought to be responsible for the hypotensive effect in both species.

A compound structurally similar to L-dopa, *m*-tyrosine (the racemic mixture and the L-isomer), has been studied in dogs and the results are herewith presented.

Methods and materials. Anaesthetized mongrel dogs of either sex, 7–11 kg, were prepared for recording arterial blood pressure and heart rate as previously described[1]. Drug preparation and i.v. administration of drugs has also been described[1]. To inhibit peripheral aromatic amino acid decarboxylase we injected carbidopa. Central inhibition of decarboxylation of aromatic amino acids was attempted with Ro 4-4602. At 100 mg/kg and higher doses, Ro 4-4602 was reported to inhibit aromatic amino acid decarboxylase in the brain of rats[6]. The hypotensive potencies of L-dopa, DL-*m*-tyrosine, and L-*m*-tyrosine were compared in anesthetized dogs pretreated with carbidopa.

Results. In control animals, there was no significant change in mean arterial pressure (MAP) or heart rate during the 2-h period (Tables I and II). DL-*m*-tyrosine by i.v. administration produced an increase in MAP. Carbidopa, 15 mg/kg i.v., or Ro 4-4602, 100 mg/kg i.v., alone had no significant effect on MAP. The pressor effect of DL-*m*-tyrosine was reversed by the decarboxylase inhibitors. A pronounced hypotensive response was observed in animals treated with carbidopa + DL-*m*-tyrosine, while only a slight hypotensive effect was produced by Ro 4-4602 + DL-*m*-tyrosine. The difference between the hypotensive responses, when comparing both groups of animals (groups 3 and 5) at all times post DL-*m*-tyrosine injection, was significant statistically ($p < 0.05$, Student's *t*-test).

In animals receiving DL-*m*-tyrosine alone, heart rate was reduced during the maximal hypertensive response. In animals receiving carbidopa + DL-*m*-tyrosine, in spite of lowered arterial pressure, the heart rate was consistently decreased to a greater extent than in control animals.

The relative hypotensive potencies of L-dopa, DL-*m*-tyrosine, and L-*m*-tyrosine in carbidopa-treated dogs were calculated as follows. Average peak effects of the drugs at each dose level were used for comparison (Table III). MAP before drug injection was recorded as the control value of each animal tested.

Preliminary evaluation revealed a correlation between peak effects and control values (i.e., low peak-effects were generally associated with low control values, and high peak-effects were generally associated with high control values). The results were adjusted, therefore, for differences in control blood pressure by analysis of covariance. DL-*m*-tyrosine was estimated to be 2.2 times more potent than L-dopa with 95% confidence limits of 1.6 and 3.1. L-*m*-tyrosine was estimated to be 4.8 times more potent than L-dopa, with 95% confidence limits of 2.6 and 11.5. The minimal hypotensive dose of L-dopa was 6.25, DL-*m*-tyrosine 3.12, and of L-*m*-tyrosine 1.56 mg/kg i.v.

Discussion. Reduction in the rate of peripheral metabolism of *m*-tyrosine by carbidopa likely increased cerebral blood concentration of *m*-tyrosine and by this mechanism may have caused increased transport of *m*-tyrosine into the brain. It is conceivable that carbidopa also diminished the enzymatic barrier (aromatic amino acid decarboxylase) said to exist between blood and brain for the

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Table I. Effect of DL-*m*-tyrosine alone and in combination with aromatic amino acid decarboxylase inhibitors on the arterial blood pressure of anesthetized dogs

Group No.	Treatment	Dose (mg/kg i.v.)	No. of animals	Control values	Mean arterial pressure (mm Hg)[a] at min after drug					
					1	5	15	30	60	120
1	Carbidopa	15	4	127	129	130	131	125	128	127
2	DL-*m*-tyrosine	25	4	149	171	233[b]	203[b]	163	137	108[b]
3	Carbidopa[c] + DL-*m*-tyrosine	15 + 25	4	122	94[b]	89[b]	65[b]	54[b]	50[b]	58[b]
4	Ro 4-4602	100	5	145	143	160	150	140	138	139
5	Ro 4-4602[c] + DL-*m*-tyrosine	100 + 25	4	161	170	170	148	140	130[b]	132[b]
6	Acidified saline control[d]		5	156	158	158	155	158	154	143

[a] Average value for 4 or 5 dogs. [b] Significantly different from control value for the same group: $p < 0.05$, Student's *t*-test. [c] Administered 5 min prior to DL-*m*-tyrosine. [d] All drugs dissolved in dilute HCl.

Table II. Effect of DL-*m*-tyrosine alone and in combination with aromatic amino acid decarboxylase inhibitors on the heart rate of anesthetized dogs

Group No.	Treatment	Dose (mg/kg i.v.)	No. of animals	Control values	Heart rate (beats/min)[a] at min after drug					
					1	5	15	30	60	120
1	Carbidopa	15	4	158	152	148	150	133[b]	136	126[b]
2	DL-*m*-tyrosine	25	4	162	128[b]	65[b]	83[b]	168	167	140
3	Carbidopa[c] + DL-*m*-tyrosine	15 + 25	4	142	130	121	85[b]	105[b]	108	100[b]
4	Ro 4-4602	100	5	160	158	163	164	160	144	123[b]
5	Ro 4-4602[c] + DL-*m*-tyrosine	100 + 25	4	145	141	150	138	140	125	115
6	Saline control[d]		5	150	150	153	153	143	132	118

[a] Average value for 4 or 5 dogs. [b] Significantly different from control value for same group; $p < 0.05$, Student's *t*-test. [c] and [d]: See Table I.

aromatic amino acid L-dopa[7,8]. If the same barrier impedes transfer of *m*-tyrosine into the brain, reduction of said barrier would favor entrance and subsequent metabolism of *m*-tyrosine since carbidopa does not enter the brain when given at the dose levels used.

Ro 460-42 at the dose level used is also capable of increasing cerebral blood concentration of *m*-tyrosine and reducing the enzymatic blood-brain barrier, but Ro 4-4602, unlike carbidopa, could have entered the brain along with *m*-tyrosine and inhibited its decarboxylation. After pretreatment of our dogs with Ro 4-4602, *m*-tyrosine had only a slight hypotensive action. It is therefore reasonable to assume that a central action of a decarboxylation product of *m*-tyrosine is responsible for the observed hypotensive effect. Centrally applied catecholamines have been shown to reduce blood pressure of cats, apparently by stimulation of α-receptors[9–11]. Reduction of arterial pressure in rats by i.p. injection of either carbidopa or HMD + DL-*m*-tyrosine has been reported by RUBENSON[12,13]. This depressor action was abolished by the dopamine β-hydroxylase inhibitor FLA-63; such abolition indicates that the depressor response may have been produced by the β-hydroxylated product of *m*-tyrosine[13].

RUBENSON[13] showed that after injection of HMD + DL-*m*-tyrosine there was a central increase in the *m*-tyrosine metabolites, *m*-tyramine and *m*-octopamine, at the time of maximal hypotensive effect; Ro 4-4602 + DL-*m*-tyrosine produced no significant reduction in arterial pressure. This further indicates that *m*-tyrosine metabolites are responsible for the hypotensive response. The lowering of arterial pressure in rats by carbidopa + DL-*m*-tyrosine did not occur following a second injection of carbidopa + DL-*m*-tyrosine, suggesting that the hypotensive effect was due to displacement of central monoamines. ANDEN et al.[14], using behavioral studies with rats, showed that treatment with *m*-tyrosine caused a stimulation of central dopamine receptors, whereas no effect on these receptors was observed after depletion of central catecholamines with α-methyl-*m*-tyrosine. This lends weight to the opinion that displacement of central monoamines is the mechanism which produced the

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Table III. Effects of L-dopa, DL-*m*-tyrosine, or L-*m*-tyrosine on mean arterial pressure of dogs when pretreated with carbidopa (15 mg/kg i.v.)

Drug	Dose (mg/kg i.v.)	No. of animals	Control mean arterial pressure (mm Hg)	Average maximum reduction in mean arterial pressure, (mm Hg)[b]
L-Dopa[a]	6.25	4	146	17
	12.5	6	137	30
	25.0	7	145	61
DL-*m*-tyrosine[a]	3.12	4	140	11
	6.25	6	141	50
	12.5	4	160	54
	25.0	4	122	74
L-*m*-tyrosine[a]	1.56	4	136	31
	3.12	4	127	40
	6.25	4	145	52
	12.5	4	155	68

[a] Administered 5 min post carbidopa injection. [b] 15–30 min after L-dopa injection; 30–60 min after DL- or L-*m*- tyrosine injection.

hypotensive effect of carbidopa + DL-*m*-tyrosine. In conclusion, our findings and the findings of others cited conclusion, our findings and the findings of others cited[2-4,9-13] imply that *m*-tyrosine produces the hypotensive effect by a central mechanism after peripheral aromatic amino acid decarboxylase inhibition. The response is likely elicited by the metabolites of *m*-tyrosine.

Résumé. Chez des chiens anesthésiés, la *m*-tyrosine donne une réponse hypertensive à l'injection i.v. Cette réponse devient hypotensive lorsque les chiens sont traités au préalable par les inhibiteurs de la décarboxylase de l'acide aminé aromatique carbidopa ou Ro 4-4602. Chez les chiens traités au préalable par le carbidopa, la L-*m*-tyrosine est considérée comme un agent plus puissant que le racémate. Comme agent hypotenseur la L-Dopa est moins puissante que la DL- ou la L-*m*-tyrosine.

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Ethanol Narcosis in Mice: Serotonergic Involvement

The possibility that biogenic amines or their respective metabolites may be involved in the soporific action of ethanol derives support from a number of investigators[1-4]. The findings of this report suggest that biogenic amine metabolites, rather than the amines per se, may be involved in the mechanism of action of ethanol. The data further imply that the observed synergistic effect of L-dopa or dopamine on ethanol-induced sleep time in mice may be due in part to a serotonergic rather than a direct, dopaminergic mechanism[5].

Ethanol narcosis was induced in 100 albino, male, Swiss-Webster mice (18-25 g) by administering 87 m*M*/kg of ethanol i.p. The measured response, sleeping time, was defined as the length of time required for each mouse to regain the righting reflex[6]. If a mouse did not recover this reflex within $2^1/_2$ h after the ethanol administration, a score of 150 min was assigned as its sleeping time.

Ethanol was combined with sodium chloride (0.9%) as a 25% v/v solution. Tryptophol was suspended in peanut oil, and all other compounds were dissolved in a saline solution. All doses are expressed in terms of the base, and injections were given i.p.

The duration of the ethanol-induced sleep response was compared for groups of mice pretreated with the saline solution or peanut oil, L-dopa, dopamine, 3,4-dihydroxyphenylethanol (DOPET), serotonin or the neutral metabolite, tryptophol, at various time intervals prior to ethanol injection. The effects on ethanol-induced sleep time after the administration of these 5 compounds, with methysergide, an antiserotonin compound[7], administered 30 min previously, were observed.

The effects of methysergide (0.28 m*M*/kg) on the synergistic action of serotonin (0.28 m*M*/kg)-dopamine (0.06 m*M*/kg)-and L-dopa (5.2 m*M*/kg)-ethanol (87 m*M*/kg)-combinations in mice are illustrated in the Figure. A significant synergistic effect ($P < 0.01$) as measured by the Student's *t*-test was observed in mice treated with methysergide and ethanol. Methysergide was found to significantly inhibit the augmentation of ethanol-induced sleep by dopamine ($P < 0.001$) and serotonin ($P < 0.001$).

The interpretation of the effect of methysergide and L-dopa on the sleep time response is a little more difficult to assess than the effects of methysergide in combination with dopamine or serotonin. L-dopa does augment the sleep time response as does L-dopa plus methysergide (41.8 ± 12.0 as compared to 32.5 ± 13.0). Upon reexamination of the Figure, it can be seen that methysergide alone augments sleep time to about the same magnitude as methysergide plus L-dopa. Thus it can be argued that

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Effects of methysergide alone and in combination with dopamine, L-DOPA, serotonin, 3,4-dihydroxyphenylethanol (DOPET) and tryptophol on blood and brain ETOH concentrations (mg/100 ml ± S.E.)

Pretreatment	Saline		Dopamine (0.06 m*M*/kg)		L-DOPA (5.2 m*M*/kg)		Serotonin (0.28 m*M*/kg)		DOPET (5.2 m*M*/kg)		Tryptophol (1.5 m*M*/kg)	
	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain
Methysergide 0.28 m*M*/kg	321.0	139.1	286.2	167.7	285.3	146.2	224.6	184.6	207.6	176.8	310.0	176.2
	±	±	±	±	±	±	±	±	±	±		±
Injected I.P. 30 min. prior	10.9	2.94	22.9	19.9	36.1	19.1	13.2	12.6	23.5	14.7	33.8	23.1

Mg/100 ml ± S.E. values for saline + ETOH were: blood = 248.0 ± 28.2 and brain = 208.5 ± 9.0. These values did not differ significantly from the saline control. All agents listed in the table were administered in combination with 87 m*M*/kg ETOH. The animals were sacrificed and the blood and brain were collected at the following average sleep times: saline, 10 min; dopamine, 116 min; L-DOPA, 45 min; serotonin, 129 min; DOPET, 124 min; tryptophol, 52 min.