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BRAIN AND VASCULAR MONOAMINE OXIDASE ACTIVITY IN THE DEOXYCORTICOSTERONE-SALT HYPERTENSIVE RAT

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1 The role of monoamine oxidase (MAO) in the maintenance of deoxycorticosterone-sodium chloride (DOCA-salt) hypertension was investigated by assaying the MAO activity both in central as well as peripheral blood vessels and in brain tissue.

2 The results suggest that the activity of MAO in the DOCA-salt hypertensive rat is similar to the activity present in the normotensive rat.

Introduction

Many workers have reported on the importance of the central nervous system in the development or maintenance of various forms of experimental hypertension, such as renal hypertension (Chalmers, Dollery, Lewis & Reid, 1974), deoxycorticosterone (DOCA)-salt hypertension (Finch, Haeusler & Thoenen, 1972), and hypertension induced by lesions of the anterior hypothalamus in the rat (Nathan & Reis, 1975). It has also been suggested (de Champlain, Mueller & Axelrod, 1969; Reid, Zivin & Kopin, 1975) that DOCA-salt hypertension causes increased peripheral adrenergic activity through a central component.

In DOCA-salt hypertension, there is an increased urinary excretion of noradrenaline (NA) and its metabolites (de Champlain *et al.*, 1969) and, in addition, the cardiac NA turnover rate is increased (de Champlain *et al.*, 1969, Nakamura, Gerold & Thoenen, 1970). Though the half-life of cardiac NA was short and more NA and its metabolites were present in the urine of DOCA-salt hypertension, cardiac catechol-*O*-methyl tranferase enzyme in DOCA-salt hypertensive rats was found to be similar to that in the controls (de Champlain, Krakoff & Axelrod, 1968). Although cardiac monoamine oxidase (MAO) was increased in the hypertensive rats, the elevation was due to the cardiac hypertrophy which occurred during the DOCA-salt treatment (de Champlain *et al.*, 1968).

Recently Reid *et al.* (1975) have shown that although the plasma NA is increased in the DOCA-salt induced hypertensive rat, there is no parallel increase in the plasma dopamine-β-hydroxylase. Since an appreciable fraction of circulating NA and NA metabolites appearing in urine may be derived from the sympathetic supply to blood vessels (Bigelow, Dairman, Weil-Malherbe & Udenfriend, 1968; Berkowitz, Tarver & Spector, 1971), and limited information on vascular MAO in DOCA-salt hypertension is available, we decided to examine the role of vascular MAO in the maintenance of DOCA-salt hypertension.

Methods

Uninephrectomized male Wistar rats (8 weeks old) obtained from Charles River Breeding Lab. Inc., Wilmington, Mass., were divided into two groups, each fed a regular laboratory diet. One group of rats was made hypertensive by the subcutaneous injection of a suspension of DOCA acetate (ICN Pharmaceuticals, Inc., Cleveland, Ohio), 5 mg/kg twice a week for a period of seven weeks, and the addition of 1% NaCl solution in their drinking water. The other group of uninephrectomized rats were allowed free access to the drinking water containing 1% NaCl but were not treated with DOCA acetate and thus served as the control group. The systolic blood pressure of the rats in both groups was measured by a tail cuff microphone method using an instrument made by Hoffmann-LaRoche & Co. Ltd., Basle, Switzerland.

Animals were killed by decapitation and their brains removed. The brain microvessels were prepared by a modification of the method of Brendel, Meezan &

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Carlson (1974), as previously described (Lai, Udenfriend & Spector, 1975). The brain filtrate was designated as that fraction of the homogenate which passed through the nylon mesh. Vascular tissue, i.e. aorta, mesenteric artery and mesenteric vein, was removed and perivascular adipose tissue carefully dissected away from the vascular tissue.

MAO activity was determined by the method of Goridis & Neff (1971) with the modification that the substrate (tyramine) concentration was increased to 2.5 mM.

All tissues were homogenized in phosphate buffer (67 mM; pH 7.2). Brain microvessels (from four brains) were homogenized in 120 µl; mesenteric arteries and veins in 20 volumes, and aortae in 15 volumes. The reaction mixture consisted of 2.5 mM tyramine containing about 8.5×10^4 ct/min of [1-^{14}C]-tyramine plus the tissue homogenate, in a total volume of 140 µl of buffer solution. The mixture was incubated for 30 min at 37°C and the reaction was terminated by the addition 40 µl of 60% perchloric acid. After centrifugation of the sample, the supernatant was passed over a Rexyn 101 column (H^+, 200–400 mesh) and washed with 5 ml of distilled water. The effluent and washes were collected and mixed with 15 ml of Aquasol in a counting vial. Radioactivity was measured in a Beckman Liquid Scintillation Counter. Tissue protein was determined according to the method of Lowry, Rosebrough, Farr & Randall (1951).

Results

After seven weeks of treatment with DOCA-salt, rats became hypertensive with systolic blood pressures (mean ± s.e., mmHg) (*n*) of 205.3 ± 5.6 (12), significantly ($P < 0.001$) higher than that of controls, 138 ± 3.6 (12). The DOCA-salt treated group also showed a reduced weight gain, final weights being 328.0 ± 12.2 g (12) compared to the control group 403.4 ± 8.2 g (12) ($P < 0.001$).

The central and peripheral vascular and brain MAO activities are summarized in Table 1. MAO activities in brain filtrate and brain microvessels of DOCA-salt hypertensive rats were not different from those of the controls. It is of great interest to note that the enzyme activity in brain microvessels in both DOCA-salt hypertensive and normotensive rats is 3.0-fold more than that of brain filtrate. The MAO activities in mesenteric arteries and veins and in aortae of hypertensive animals were found to be similar to those of the control but not as great as the activity found in brain microvessels. It also can be seen from the table that MAO activity in mesenteric vein is approximately two-fold that of mesenteric artery or aorta in both the hypertensive and control groups.

Discussion

MAO is the enzyme responsible for the oxidative deamination of various biogenic amines found in many tissues. MAO also plays an important physiological role by controlling the level of intracellular monoamine at nerve terminals (Kopin, 1964). The presence of this degradative enzyme in blood vessels such as the mesenteric artery, mesenteric vein and aorta has been demonstrated elsewhere (Lai, Berkowitz & Spector, 1975). Our results show that the MAO activity in the vascular and brain tissue of the hypertensive animal is not different from the control. This would imply that the increased plasma NA observed during the development of DOCA-salt hypertension (Reid, *et al.*, 1975) is not due to the impairment of the MAO catalytic system in vascular tissues.

We have reported (Lai, Berkowitz & Spector, 1976) that following the inhibition of brain microvessels MAO by clorgyline, intraventricular injection of NA produces a profound pressor effect. We, therefore, proposed that the brain microvessel MAO might function to inactivate that NA which is synthesized in the central nervous system. It would thus prevent the NA from impinging on sites in the brain which might elicit hypertension. Additionally, it could perhaps prevent the efflux of NA to peripheral sites.

Table 1 Monoamine oxidase (MAO) activity in various tissues of normotensive and DOCA-salt hypertensive rats

	MAO activity (nmol mg^{-1} protein h^{-1})	
Tissues	*Normotensive*	*Hypertensive*
Brain filtrate	133.4 ± 9.6 (3)	130.2 ± 17.2 (3)
Brain microvessels	408.8 ± 45.9 (3)	399.4 ± 56.8 (3)
Mesenteric artery	63.6 ± 5.6 (7)	68.9 ± 5.3 (7)
Mesenteric vein	130.6 ± 11.3 (6)	115.9 ± 8.4 (6)
Aorta	57.3 ± 3.4 (6)	60.3 ± 3.1 (6)

Blood vessels were pooled and homogenized in phosphate buffer (67 mM, pH 7.2) for each experiment. Brain microvessels, from four brains, were homogenized in 120 µl phosphate buffer, mesenteric artery and vein were homogenized in 20 volumes of the buffer while aorta was homogenized in 15 volumes of buffer. Brain filtrates were collected from four grains and centrifuged at 120 *g* for 10 min at 4°C. The brain precipitate was then resuspended and homogenized in 150 ml of phosphate buffer and an aliquot of that was used for MAO assay. Figures represent mean ± s.e. Numbers in parentheses refer to the number of experiments.

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