ESTERASES OF THE AORTIC WALL IN RATS WITH DOCA-SALT AND RENAL HYPERTENSION

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Esterases of the aortic wall in rats with DOCA-salt and renal hypertension were studied by electrophoresis in polyacrylamide gel. Material from rats with hypertension and from the control showed five zones of esterase activity. Esterases of the aortic wall from rats with DOCA-salt and renal hypertension characteristically showed a relative decrease in activity in zone 2 from the start and an increase in activity in zone 5. In rats with DOCA-salt hypertension there also was a relative decrease in esterase activity in zone 1. In rats receiving DOCA and salt but remaining normotensive, the relative increase in activity of esterases in zone 5 was less marked than in rats with DOCA-salt hypertension.

It was shown previously that in renal [4] and DOCA-salt hypertension [2] activity of nonspecific esterase is low in the media and endothelium of the rat aorta. However, these histochemical observations reflect only the total activity of a group of enzymes hydrolyzing a common substrate, and they do not allow the state of the individual enzymes of the group to be assessed. Nevertheless, the esterases of blood vessel walls, which on the whole have received very little study, are interesting not only because of their role in the lipid metabolism of the arterial wall in general, but also because of their direct concern with metabolism of the phospholipid components of the membranes of its smooth-muscle and endothelial cells. This accounts for the great importance of a study of the vessel wall as the substrate in which all the pathogenic factors producing hypertension and ultimately producing a stable elevation of the arterial pressure are concentrated.

In the investigation described below electrophoresis in polyacrylamide gel was used to determine which components of the esterase system of the arterial wall are modified in these forms of arterial hypertension.

EXPERIMENTAL METHOD

The 23 male Wistar rats weighing 180-230 g used in the experiments were divided into three groups.

The animals of group 1 (11 rats) received a combination of treatments leading to the development of DOCA-salt hypertension [5]. Unilateral nephrectomy was performed on the animals, they were given 1% sodium chloride solution to drink instead of water, and tablets containing 100 mg deoxycorticosterone acetate (DOCA; Organon, Holland) were implanted subcutaneously. Implantation of this dose of DOCA was carried out on 3 subsequent occasions at intervals of 10 days (so that each rat received 4 tablets each containing 100 mg DOCA). The rats of this group were sacrificed at the 8th week of the experiment. Six animals giving a steady increase in arterial pressure after the 4th week of the experiment, and with a systolic pressure level of 150-170 mm Hg at the time of sacrifice, were chosen for investigation. The other five animals were resistant to the treatment and maintained a normal level of arterial pressure throughout the experiment (between 80 and 100 mm Hg).

In the six rats of group 2 ischemic renal hypertension was produced by applying a metal coil to the left renal artery [1]. The contralateral kidney was preserved. The rats of this group had stable hypertension at a level of 135-170 mm Hg for 5 months until the time of sacrifice.

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TABLE 1. Activity of Esterases (M ± m) of Different Zones of Chromatograms of Material from Rats Receiving DOCA and Salt, Rats with Renal Hypertension, and Control Rats (in percent)

Group of rats	Characteristics of experiment	Number of rats	Systolic pressure (in mm Hg)	Zone of chromatogram 1-я	2-я	3—4-я	5-я
1.	Treatment with DOCA and salt: rats with hypertension	6	159 ± 3,8	6,8 ± 1,6 <0,01	69,6 ± 1,9 <0,05	7,98 ± 1,8 >0,05	15,8 ± 0,77 <0,01
	Rats with normal arterial pressure	5	90 ± 4,4	7,76 ± 0,9 <0,01	72,3 ± 0,8 >0,1	7,36 ± 0,5 >0,05	12,4 ± 0,8* <0,05
2.	Ischemic renal hypertension	6	146 ± 4,8	12,6 ± 1,3 <0,5	68,3 ± 1,4 0,05	6,8 ± 0,4 >0,05	12,2 ± 1,06 <0,05
3.	Intact rats	6	80 ± 4,3	11,96 ± 0,6	74,5 ± 1,0	5,75 ± 0,26	8,25 ± 0,97

* Statistically significant difference between rats with DOCA–salt hypertension and rats resistant to this treatment.

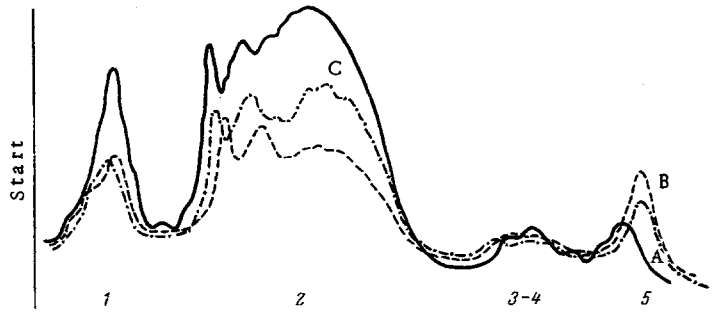

Fig. 1. Distribution of esterase activity by zones on chromatograms in DOCA hypertension: A) intact rat; B) DOCA hypertension (arterial pressure 160 mm Hg); C) animal resistant to treatment with DOCA and salt and with normal arterial pressure.

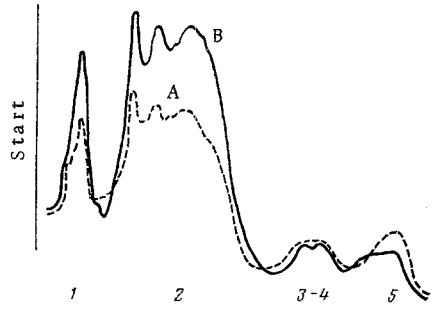

Fig. 2. Distribution of esterase activity by zones on densitograms of material from rat with ischemic renal hypertension (A) and intact rat (B).

Group 3 (control) consisted of six intact rats with a systolic pressure of 70-95 mm Hg.

All animals received food in pellet form, and the rats of groups 2 and 3 were given tapwater ad lib. to drink.

The arterial pressure was measured on the average once every 10 days. Measurements were made on the tail by recording pulsation of the caudal artery and the pressure in the cuff on a "Galileo" apparatus with photorecording. (The R 36^{z-m} myographic unit with photoelectric sensor and the R 70^{z} sphygmo-oscillographic unit were used to record the pressure in the cuff.)

The extract of the aorta was prepared, and electrophoresis in polyacrylamide gel carried out by the method of Shekhonin and Polyakova [3]. A 0.05 M tris-glycine buffer, pH 8.3, was used for electrophoresis. To detect esterase activity in the polyacrylamide gel an incubation medium containing 25 mg α-naphthyl acetate + 50 mg fast blue BB (G. Gurr, England) + 50 ml 0.1 M phosphate buffer, pH 7.4, was used. Extracts of animals of the control and experimental groups were subjected to electrophoretic fractionation simultaneously. The "Chromoscan" microdensitometer with graphic recording (Joyce and Loebl, England) was used to analyze the enzymes. The contribution of the individual fragments was calculated in percentages of the overall reading of the instrument for each chromatogram. The results of the analysis are given in the table.

EXPERIMENTAL RESULTS

The chromatograms revealed five zones of esterase activity, in agreement with the results obtained by Shekhonin and Polyakova [3]. All these fractions belonged mainly to the media of the aorta and, to a lesser degree, to its intima, for the adventitia of the aorta had been carefully removed.

Although the method used did not allow the absolute level of activity of the individual components of the esterase system to be assessed, analysis of the relative percentages of the individual fractions of esterase activity showed significant differences between the esterase spectrum of the aortic wall in rate with the two types of hypertension and in the control.

As Table 1 shows, in the rats receiving DOCA and salt the relative esterase concentration in zone 1 of the chromatogram was reduced, both in rats with a high arterial pressure and in the normotensive rats of this group (Fig. 1).

The relative concentration of esterases in zone 2 of the chromatogram was reduced both in rats with DOCA hypertension and in animals with renal hypertension (Figs. 1 and 2). Considering that normally the esterases of this zone account for more than 70% of the total activity, this suggests that the decrease in non-specific esterase activity observed in earlier histochemical investigations was largely connected with changes in the esterase activity in that zone.

In zones 3-4 of the chromatogram, which were not always completely separated, no statistically significant differences were found. The clearest changes in the distribution of esterases of the aortic wall in the two forms of arterial hypertension were found in zone 5, where the esterase activity was significantly higher than in the control. The relative increase in esterase activity in this zone in animals with DOCA–salt hypertension in these experiments correlated with the development of the arterial hypertension: in rats with hypertension the relative value of esterase activity in zone 5 was higher than in the normotensive rats of this group.

The characteristic distinguishing feature of the enzyme spectrum of the aortic wall esterases in both types of hypertension was thus a relative decrease in their activity in zone 2 and an increase in zone 5.

Investigation of the aortic wall esterases on the chromatogram with the aid of various inhibitors [3] shows that the esterases of zones 1 and 2 are cholinesterases (pseudocholinesterases), while the more mobile fractions in zones 3 and 4 are aryl esterases.

The significance of the changes in the esterase system of the arterial wall described above and the role of this factor in the pathogenesis of the forms of hypertension investigated are not yet clear. However, the comparison of these changes with the well known fact that the electrolyte balance is disturbed in DOCA–salt and renal forms of hypertension suggests that they reflect changes in lipid metabolism of the cell membranes, especially metabolism of phospholipids concerned with ion transport through the membrane.

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