DETECTION OF ESTERASES IN THE AORTIC WALL OF RATS BY ELECTROPHORESIS IN POLYACRYLAMIDE GEL

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By electrophoresis in polyacrylamide gel, 5 zones of esterase activity, comprising a total of 9-14 esterase fractions, were detected in the aortic wall of rats. Esterases of the 1st and 2nd zones were cholinesterases, and those of the 3rd-5th zones arylesterases.

Many aspects of metabolism in the vascular wall and of the regulation of vascular tone still remain unexplained. The study of enzyme activity in the vascular wall is therefore of considerable interest.

The object of this investigation was to study the content of esterases in the aortic wall of rats, because this large group of enzymes, with a very broad spectrum of action, is concerned in particular with the transmission of nervous impulses and with lipid metabolism in cell membranes.

EXPERIMENTAL METHOD

The aortic wall of noninbred male rats, kept on an ordinary diet (50 observations), was studied. The animals were killed by decapitation always at the same time of day, and the aorta was removed immediately after sacrifice. After removal of the adventitia, the aorta was washed with distilled water and homogenized. The product was extracted in 0.05 M tris-glycine buffer at the rate of 0.6 ml buffer per 100 mg aorta for 12 h at 4°. After extraction, the homogenate was centrifuged for 30 min at 8000 rpm and the supernatant was subjected to electrophoresis.

Electrophoresis was carried out at 8°C in a vertical polyacrylamide gel cuvette (200 V, 40 mA). To obtain better fractionation of the esterases, the cuvette was filled with two solutions for polymerization containing different concentrations of acrylamide. The lower gel, 75 mm high, contained 7% acrylamide, and the upper gel, 10 mm high, 6%. Both gels were made up in 0.05 M tris-glycine buffer. The upper solution for polymerization was poured as the top layer above the recently poured lower layer. Tetramethylenediamine was used to initiate polymerization and ammonia persulfate as catalyst. Some workers [4, 3] state that artefacts can arise during electrophoresis of enzymes in polyacrylamide gel due to the action of ammonium persulfate. To prevent this, before the wells were filled with aortic extracts, the ammonium persulfate was removed from the gel electrophoretically, by applying a voltage of 300 V for 30 min.

Esterases were detected by incubating the gel for 1 h at 4°C in the following solution: 25 mg substrate + 50 mg diazonium salt + 50 ml 0.1 M phosphate buffer, pH 7.4. The substrates were α-naphthylacetate, naphthol AS-acetate, and naphthol AS-D-acetate. The following diazonium salts were used: fast blue RR, fast blue B, fast blue BB, fast garnet DBC, and fast red TR.

The following inhibitors were used to indentify the esterases: eserine, p-chloromercuribenzoic acid, cupric chloride. Incubation with the inhibitors was carried out before electrophoresis (for 1 h at 20°C), or the inhibitor was added to the solution of substrate with diazonium salt.

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EXPERIMENTAL RESULTS

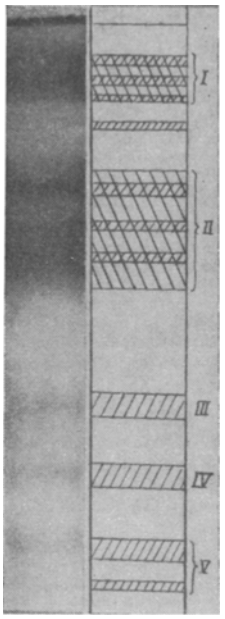

Fig. 1. Electrophoretic fractionation of esterases in aortic wall of rat (α-naphthylacetate + fast blue RR). Here and in Figs. 2 and 3: I-V) zones of esterase activity.

The clearest fractionation of the enzymes was obtained by the use of α-naphthylacetate as substrate and by treatment with fast blue RR and fast blue BB. Four or 5 zones of esterase activity were distinguished electrophoretically in the aortic extracts (Fig. 1). The 1st zone was close to the origin and confined to the upper gel and it consisted of 3 indistinctly outlined but very deeply stained bands. In some cases, an additional, 4th esterase fraction, very weak and diffuse, could be observed immediately below them (Fig. 1, I).

The 2nd zone consisted of a wide, very deeply stained band, in which 2 or 3 areas of stronger activity could be distinguished (Fig. 1, II).

The 3rd zone of esterases consisted of a blurred, weakly stained, broad band (Fig. 1, III). In some cases, however, 3 fractions could be distinguished in it.

The 4th zone (Fig. 1, IV) was weakly stained, and in half of the cases investigated it either could not be detected at all or it was merged with the 3rd zone.

The 5th zone of esterase activity of the rat aorta, the farthest from the origin, was close in its mobility to the serum albumins, and consisted usually of 1 fraction. Its activity varied, and in some cases an additional band could be observed in this zone (Fig. 1, V).

The use of naphthol AS- and naphthol AS-D-acetates as substrates gave much inferior results (Table 1). Staining was observed only in the 1st and 2nd zones, and it was much weaker than when α-naphthylacetate was used.

The addition of eserine to the incubation in a concentration of 10^{-3} M completely inhibited the activity of the 1st zone of esterases and partially inhibited the activity of the 2nd zone (Fig. 2). It was now possible to detect 4 fractions in the 2nd zone which were more resistant to the action of the inhibitor. In a concentration of 10^{-4} M, eserine did not completely inhibit the activity of the 1st zone and produced only a slight change in activity of the 2nd zone of esterases. The zones 3-5 remained completely unchanged in activity after treatment with eserine in the concentrations specified. If p-chloromercuribenzoic acid in concentrations of 10^{-3} M was used as an inhibitor, the activity of the 5th zone was completely inhibited and that of the 3rd and 4th zones partially inhibited, while the esterase activity in the 1st and 2nd zones was unchanged. In a concentration of 10^{-4} M, this inhibitor had a similar, but less marked, effect.

Cupric chloride, in concentrations of 10^{-2} and 10^{-3} M, also completely inhibited the activity of the 5th zone and almost completely inhibited the activity of the 3rd and 4th zones when incubated with the extract for 1 h (Fig. 3).

By electrophoresis in polyacrylamide gel, using α-naphthylacetate as substrate, 5 zones of esterase activity were thus revealed in the rat aorta. Activity was strongest in the 1st and 2nd zones, with low and average electrophoretic mobility. Subdivision into fractions was observed in almost every zone, and the total number of fractions in the different aortas was 9-14.

TABLE 1. Detection of Esterases in Aortic Wall of Rat by means of α-naphthylacetate and Naphthol AS- and Naphthol AS-D-acetates and Various Diazonium Salts

Electrophoretic zones of enzyme activity	Naphthol AS-acetate					Naphthol AS-D-acetate					α-Naphthylacetate				
	blue RR	blue B	blue BB	garnet DBC	red TR	blue RR	blue B	blue BB	garnet DBC	red TR	blue RR	blue B	blue BB	garnet DBC	red TR
I	++	++	++	++	+	+	++	++	+	−	+++	+++	+++	+++	+++
II	++	++	++	++	+	+	++	++	+	−	+++	+++	+++	+++	+++
III	−	−	−	−	+	+	+	−	−	−	++	++	++	++	++
IV	−	−	−	−	+	+	+	−	−	−	++	++	++	++	+
V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

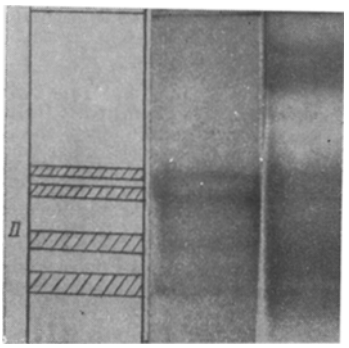

Fig. 2. Inhibitory action of eserine (10^{-3} M) on esterase activity in the 1st and 2nd zones. On left: action of inhibitor, 4 fractions relatively resistant to the action of eserine are revealed. On right: control (α-naphthylacetate + fast blue RR).

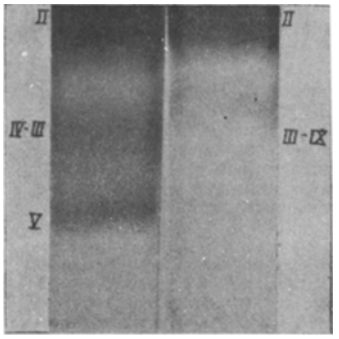

Fig. 3. Inhibition of esterase activity in 3rd-5th zones of cupric chloride in concentration of 10^{-3} M. On left: action of inhibitor; on right: control (α-naphthylacetate + fast blue RR).

By treatment with different inhibitors, an attempt was made to identify the esterases thus discovered, in accordance with Augustinsson's classification [2]. Many workers have described the selective inhibitory action of eserine on cholinesterases [1, 2, 7]. Because of the inhibitory effect of eserine on the esterases of the 1st and 2nd zones, and because of their low electrophoretic mobility, these esterases were regarded as belonging to this group.

No differential analysis was made of the content of pseudocholinesterase and acetylcholinesterase in the rat aorta. Mainly pseudocholinesterase (90%) is found in the rabbit aorta, while acetylcholinesterase (10%) is localized only in the nerve endings of the adventitia [6]. Similar results have been obtained by histochemical investigation of the rabbit and rat aorta [5]. The fractionation and the relative content of the cholinesterases in the material examined in the present investigation were evidently analogous, and the cholinesterases observed belonged to the pseudocholinesterase group.

The 3rd, 4th, and 5th zones of esterase activity of the aorta were completely insensitive to eserine but were inhibited by cupric ions and by p-chloromercuribenzoic acid. Since these properties belong to the arylesterases only [7], it is evident that the esterases of the 3rd-5th zones belonged to this group.

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