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EFFECT OF PYRIDINOLCARBAMATE ON ENZYME CHANGES IN THE ARTERIAL WALL OF RATS INTOXICATED WITH ALLYLAMINE

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Abstract—Parenteral administration of allylamine results in changes in activity of some important enzymes of basic metabolic cycles in the arterial walls of the rat. Administration of pyridinolcarbamate at a dose of 25 mg/kg per day for 3 weeks prevented the decrease in activity of aortic LDH and MDH which is characteristic of vascular wall damage. Anaerobic fractions of LDH, which are increased after allylamine intoxication, are unchanged when pyridinolcarbamate is also administered.

It is suggested that endothelial damage of the vascular wall is the predominant factor in the development of atherosclerosis and its complications [1–4]. The damage to the vessel wall has an effect on metabolism, which is manifest mainly in a decrease in the activity of important enzymes of the Krebs cycle and glycolysis with an increase in the activity of some phosphomonoesterases [5–7].

Processes involved in inhibiting acute oedematous changes in the arterial wall would appear to be an essential factor in the prevention of atherosclerosis [8]. Previous studies have shown that pyridinolcarbamate [2,6-pyridin-dimethanol-*N*-methylcarbamate (PDC), Pharmaceutical Banyu Co.] is a potent substance capable of preventing and abolishing oedema of the arterial wall [9, 10]. As opposed to classical drugs, PDC does not have any hypolipaemic activity, and recent electron microscopic studies by Shimamoto *et al.* have shown that PDC significantly inhibits contraction and "blebbing" of endothelial cells, i.e. a process which could be one of the causal mechanisms producing atherosclerosis [11]. Our own previous work has shown that PDC has a favourable effect on the metabolism of the vessel wall under conditions promoting lipid accumulation and arterial lesions in experimental animals [12, 13].

An effective and selective agent producing inflammation and necrosis in the arteries of experimental animals is allylamine [14, 15]. Since parenteral administration of this substance results in marked changes in the activity of crucial enzymes of energy metabolism, we have attempted to investigate the effect of PDC on vessel wall metabolism under conditions of this model of vascular damage.

METHODS

Fifty-five female Wistar stain rats, average body wt 200 g, were used in these experiments. Allylamine (Schuchard), as a 1% solution neutralised with HCl, was injected into the tail vein of 40 rats at a dose of 20 mg/kg twice-weekly for 3 weeks. Half of this group received, by gastric intubation, 25 mg/kg pyridinolcarbamate, as a suspension in tap water, daily for 1 week before the first injection of allylamine. Administration of PDC then continued daily for the remaining 3 weeks of the experiment. The control group (15 animals) received tap water in the same manner and physiological saline was injected i.v. twice weekly. The experiments were terminated by decapitating the animals and aortae were removed for biochemical, histological and histochemical analysis. For biochemical determinations, the vessels were cleared of periaortic tissue and adventitia and from each vessel segment, using an ultrahomogeniser (Ultraturax we prepared either a 1% homogenate in 0·9% NaCl, pH 7, for determining enzyme activities, or a 10% homogenate in veronalacetate buffer pH 8·6 for electrophoretic separation of LDH isoenzymes. The homogenate was kept at 0–3° for one hour before being centrifuged at 1000 *g*; the supernatant was used for analysis. Since an increased activity of some phosphomonesterases and a decreased activity of enzymes of the Krebs cycle or of glycolysis are characteristic features of arterial wall damage, we measured the activity of the following enzymes: acid phosphatase (ACP, EC 3.1.3.2), determined by a modification of the method of Kaplan and Narahara, and malate dehydrogenase (MDH, EC 1.1.1.37) and lactate dehydrogenase (LDH, EC 1.1.1.27), determined by the neotetrazolium method using phenazinemetosulphate

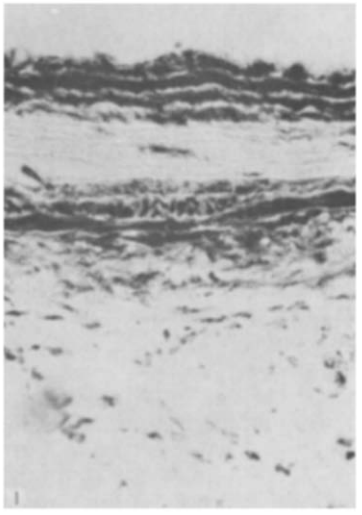

Fig. 1. The middle part of the aortic media with negative activity of MDH after treatment with allylamine and without PDC. MDH, 270×.

as a direct electron acceptor (for details see [16, 17]). LDH isoenzymes were separated by electrophoresis on W_3 paper, in barbital buffer pH 8·6 at a voltage drop of 6 V/cm for 4 hr. The separated fractions were eluted with acetone and the optical density determined on an Sf 4 (Optica-Milano) spectrophotometer at 490 nm. The relative values of the LDH fractions were used to calculate the percentage distribution of separate fractions in the total enzyme sample [18].

The activity of the enzymes investigated were expressed on a protein basis, the protein content of the extract being determined by the method of Lowry *et al.* [19]. The data were evaluated statistically using Student's *t*-test.

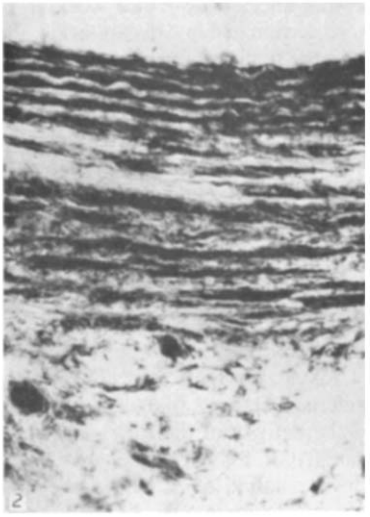

Fig. 2. The negative activity of MDH is smaller after treatment with allylamine and PDC. MDH, 270×.

RESULTS

Histological controls showed that aortic medial oedema and the cellular reaction of the adventitia could be detected in the aortae of allylamine-treated rats. The more advanced lesions showed areas of medial necrosis and a more intensive adventitial cellular reaction. In animals treated with allylamine and PDC, necroses were also found, but they were of significantly lower extent than in rats treated with allylamine alone. The enzymatic pattern of these necroses was the same in both experimental groups (Figs. 1 and 2).

After parenteral administration of allylamine there was a significant increase in acid phosphatase activity even in the aortae of animals which also received PDC (Fig. 3). On the other hand, administration of PDC prevented the decrease in the activity of aortic MDH and LDH, which are considered to be characteristic indicators of vessel wall damage [5–7] (Fig. 3).

The zymogram of LDH shows (Fig. 4) that in the aortae of experimental animals there was an increase in the anaerobic fraction and a decrease in the aerobic fraction, both in the group treated with allylamine alone and in the group treated with allylamine and PDC. Quantitative evaluation of the zymograms showed that in the group with allylamine intoxication and simultaneous administration of PDC, the separate LDH fractions, particularly the anaerobic fraction, had values similar to those in control animals (Fig. 5). This means that PDC prevented the increase in anerobic fractions of LDH which occurred in animals treated only with allylamine.

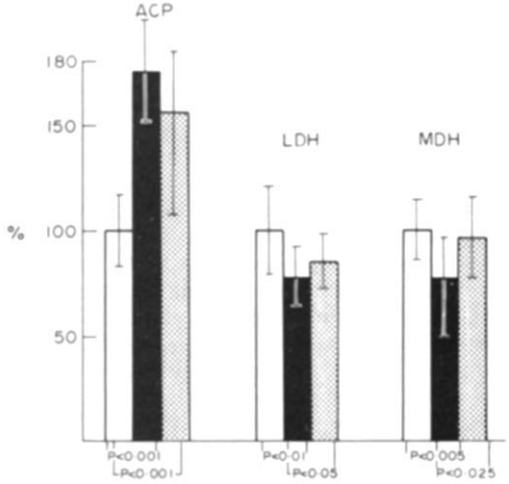

Fig. 3. The effect of PDC on aortic acid phosphatase (ACP), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in rats intoxicated with allylamine. Empty columns, control rats; black columns, allylamine-treated rats; hatched columns, allylamine- and PDC-treated rats. (Average activity in the aortae of control rats: ACP, 1·02 μmoles of phenol/100 μg protein/hr; LDH, 182·3 μg diformazan/100 μg protein/hr; MDH, 70 μg diformazan/100 mg protein/hr). Results are expressed as percentage differences, taking the mean activity of controls as 100% (mean ± S.D.).

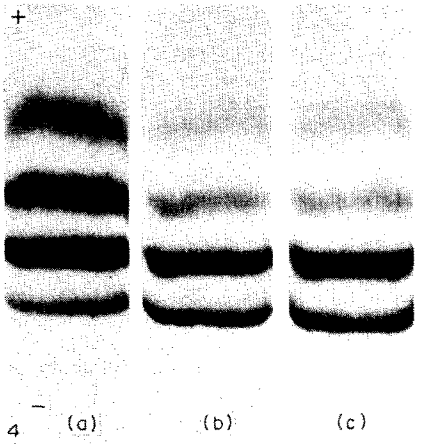

Fig. 4. Representative picture of electrophoretic separation of LDH isoenzymes in the aortae of normal (A), allylamine-treated (B) and allylamine- and PDC-treated rats (C).

DISCUSSION

As reported previously [12, 13] the administration of pyridinolcarbamate does not influence the increase in activity of aortic acid phosphatase. Since the increase in activity of this enzyme is associated with an intensification of metabolism of connective tissue in the damaged vessel, it is apparent that PDC does not interfere with the metabolism of connective tissue. Allylamine disturbs the plasma membrane and its administration is related to an intensification of the metabolism of connective tissue in the damaged vessel, so that it is felt that PDC plays no role in the metabolism of connective tissue. Allylamine breaks down plasma membranes [20] and its administration is accompanied by a decrease in the activity of Krebs cycle and glycolytic enzymes. Since energy metabolism of the vessel wall is relatively inefficient (51 per cent from glycolysis and 49 per cent from respiration) and total production of energy [21] requires a relatively high glucose utilisation, even small alterations in the efficiency of oxidative phosphorylation and Krebs cycle enzymes may be disadvantageous to the energy balance in the vessel wall. The effect of pyridinolcarbamate, which prevents the decrease in MDH and LDH activities associated with vessel wall damage, can therefore be considered to be beneficial to the organism.

Under conditions of hypoxia, which is involved in damage of the vessel wall, anaerobic glycolysis takes on a more important role as an energy supplier. In the damaged aorta the anaerobic fraction of LDH is increased, i.e. the fraction which is stimulated by a low oxygen tension [22] and the biological function of which is to maintain LDH activity even in the presence of an excess of lactate. Increased lactate production results in acidosis, which can be considered to be a pathogenetic factor in atherosclerosis [23]. Electron-microscopic studies have shown that under the effect of a decreased pH (to pH 4·2) there is an opening of endothelial junctions and a breakdown of cell membranes [20]. This disadvantageous situation, from the point of view of energy, was inhibited in the case of allylamine intoxication by simultaneous administration of PDC, manifest mainly in the fact that aortae of animals treated with both allylamine and PDC did not show a marked shift between the separate fractions of LDH towards anaerobic fractions, as in the case with administration of allylamine alone.

These results are a continuation of our previous work on the metabolic effects of various diets and calcipherol intoxication [12, 13] and are in agreement with histoenzymatic studies which show the action of PDC on arterial metabolism [24]. The results obtained are not easy to interpret from the point of view of the mechanism involved. The relationship between the metabolism of the vessel wall and the contraction of endothelial cells i.e. with that mechanism which appears to be of primary importance in the origin of atherosclerosis is not yet apparent [11].

In any case, PDC protects against "swelling" of endothelial cells which is accompanied by a dissociation and an influx of plasma into the vessel wall [25]. This permeability-inhibiting effect of PDC is probably related to its biologically advantageous effect upon the metabolism of damaged vessels.

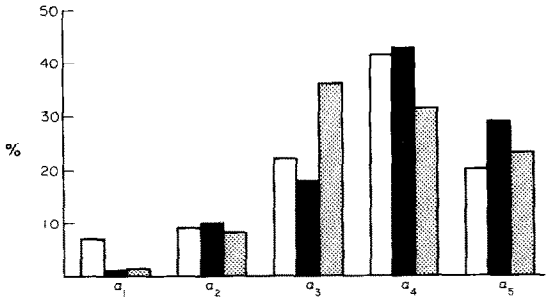

Fig. 5. Individual LDH fractions in the normal (empty columns), allylamine-treated (black columns) and allylamine- and PDC-treated rats (hatched columns), as shown in Fig. 4. The sum of all LDH fractions was taken as 100 per cent.

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