# EFFECT OF PANTHETINE ON POSTHEPARIN LIPOLYTIC ACTIVITY AD LIPID PEROXIDATION IN THE MYOCARDIUM

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Antioxidants are polyfunctional compounds which can participate in reactions with free radicals, interact with cell receptors, become inserted into biomembranes, modifying their structure and function, and act on enzymes, changing their activity [8]. A substance of interest in this connection is panthetine, a CoA precursor which, besides an antioxidative [1], also has a hypolipidemic action [12, 15]. The mechanism of the hypolipidemic action pi panthetine has not yet been explained [2, 6]. Panthetine is known to modify the blood lipoprotein (LP) spectrum and an important step in LP metabolism is lipoprotein lipolysis.

The aim of the present investigation was to study the effect of panthetine on activity of enzymes determining lipoprotein lipase activity. To study this problem we investigated the action of panthetine on the postheparin lipoprotein lipase activity (PHLA) of the plasma, lipoprotein lipase (LPL) activity, hepatic endothelial lipase (HEL) activity, and also the antioxidative activity of panthetine.

## EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 210-250 g. The experimental animals received an intramuscular injection of panthetine [D-*bis*(N-pantothenyl-$\beta$-aminoethyl)disulfide; from "Daichi," Japan] in concentrations of 5, 10, 15, 25, and 50 mg/kg 24 h before decapitation. Heparin solution was injected into the caudal vein of all the animals in a dose of 2.5 U/kg body weight. The animals were decapitated 5 min after heparinization. Blood was taken into test tubes with 0.25 ml of a 2.5% solution of trisodium citrate. To determine postheparin lipolytic activity, the samples were incubated in 200 mM Tris-HCl buffer, pH 8.6, containing 5.4% albumin solution (0.75 ml) and 0.05 ml of 20% lipofundin ("Leiras"-20, West Germany), and 0.2 ml plasma, at 37°C for 1 h. The incubation mixture for determination of HEL activity also contained NaCl (0.25 g/5.0 ml). LPL activity was determined as the difference between PHLA and HEL activity [4]. The concentration of nonesterified fatty acids (NEFA) was determined from their conversion into copper salts, which were extracted from the aqueous phase into the organic phase, where the copper content was determined [10]. Mitochondria were isolated from the myocardium by differential centrifugation [9]. Malonic dialdehyde was estimated as in [7]. The protein concentration was determined by the biuret method.

## EXPERIMENTAL RESULTS

In a dose of 5 mg/kg panthetine raised the plasma PHLA by 60.6% and LPL activity by 39.9% (Table 1). Thus the rate of hydrolysis of triacylglycerides (TG) and also to some extent of phospholipids (PL), mainly chylomicrons and very low density lipoproteins (VLDL), was increased. Activity of HEL, which hydrolyzes TG and PL of lipoproteins with intermediate density, was increased by a lesser degree. Investigations of the blood lipids showed that panthetine, injected in a dose of 5 mg/kg, lowered the plasma total lipid level by 18.3%, the TG level by 30%, and the VLDL fraction by 52.1%, while at the same time raising the

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TABLE 1. Effect of Panthetine on Postheparin Lipolytic Activity of Plasma and Lipoprotein Lipase Activity (M ± m)

| Group of rats | Dose of panthetine, mg/kg | PHLA, mg NEFA/ml/h | LPL activity, mg NEFA/ml/h |
|---|---|---|---|
| Heparinized rat | — | 16,0±3,7 (9) | 14,3±3,5 (9) |
| The same | 5 | 25,7±3,5* (8) | 20,0±3,7 (8) |
| « » | 10 | 19,6±2,6 (8) | 12,4±1,7 (8) |
| « » | 15 | 14,4±1,7 (10) | 10,2±1,2 (10) |
| « » | 25 | 13,7±2,3 (9) | 9,0±0,9 (8) |
| « » | 50 | 12,3±2,6 (7) | 7,2±1,3 (6) |

**Legend.** Here and in Tables 2 and 3, number of animals shown in parentheses.

*$p < 0.05$ compared with control.

TABLE 2. NEFA Concentration in Plasma, and in Myocardial Homogenate and Mitochondria after Administration of Panthetine (M ± m)

| Group of rats | Dose of panthetine, mg/kg | NEFA, mg/ml plasma | NEFA, mg/mg protein in homogenate | NEFA, mg/mg protein in mitochondria |
|---|---|---|---|---|
| Heparinized rat | — | 0,31±0,06 (15) | 0,98±0,08 (8) | 0,94±0,14 (5) |
| » » | 5 | 0,28±0,04 (11) | 1,65±0,16* (9) | 0,63±0,13* (6) |
| » » | 10 | 0,30±0,05 (12) | 0,94±0,09 (10) | — |
| » » | 15 | 0,22±0,05 (13) | 0,79±0,08 (10) | 0,85±0,09 (6) |
| » » | 25 | 0,24±0,04 (12) | 0,78±0,01 (8) | — |
| » » | 50 | 0,24±0,06 (11) | 0,71±0,07 (6) | 0,78±0,06 (6) |

TABLE 3. MDA Concentration in Myocardial Mitochondria After Injection of Various Doses of Panthetine (M ± m)

| Group of rats | Dose of panthetine, mg/kg | MDA, nmoles/mg protein |
|---|---|---|
| Heparinized rat | — | 1,14±0,27 (8) |
| » » | 5 | 0,66±0,24* (6) |
| » » | 15 | 0,94±0,33 (8) |
| » » | 50 | 0,96±0,20 (8) |

PL level by 27.0%. Lowering of the hyperlipidemia and the beneficial effect on the ratio of VLDL and LDL to HDL have been confirmed by a clinical study of panthetine [14] and also experimentally [2]. Injection of panthetine in a dose of 10 mg/kg increases PHLA by 22.5%. Higher doses of panthetine do not possess this action. The results relating to the increase in PHLA after injection of panthetine are in agreement with data obtained by Japanese workers [11], who showed in experiments on rats that PHLA is increased after administration of panthetine in low concentrations (2-7 mg/kg). Lowering of the LDL level with a parallel increase in concentrations of HDL fractions was observed.

To study correlation between PHLA and the NEFA level we determined the NEFA concentration in the plasma. We also studied the action of panthetine on the plasma catecholamine level, for increased catecholamine secretion can induce elevation of the blood NEFA level through intensified lipolysis in adipose tissue. However, we found neither elevation of the catecholamine level nor an increase in the NEFA concentration in the plasma after injection of panthetine in doses increasing PHLA (Table 2). Considering that as a result of lipoprotein lipolysis, the released NEFA are quickly extracted by the myocardium, which is perfectly realistic because a comparatively large fraction of LPL is located on the surface of the cardiac capillaries [5], we determined the NEFA concentration in myocardial homogenate. The results showed that after injection of panthetine in a dose of 5 mg/kg the NEFA concentration in the homogenate was considerably increased (88.0%). According to data in the literature, myocardial neutral lipase is hormone-sensitive and is involved in intracellular lipolysis in the heart [13]. Consequently, it cannot play an essential role in the elevation of the myocardial NEFA level, for no increase was found in the catecholamine concentration after injection of panthetine. Since fatty acid biosynthesis virtually does not take place in the myocardium, it can

be tentatively suggested that elevation of the NEFA level in the homogenate is the result of increased LPL activity after administration of panthetine (5 mg/kg). In doses of 15, 25, and 50 mg/kg panthetine did not have this effect. The above-mentioned concentrations actually lower the NEFA level in the myocardium, in agreement with the decrease in the plasma PHLA associated with these concentrations of panthetine.

The picture concerning the NEFA level was completely different in the myocardial mitochodnria. A dose of panthetine of 5 mg/kg, which increases PHLA, LPL activity, and NEFA accumulation in the homogenate, reduces their concentrations in the mitochondria. In our view this is evidence of intensive NEFA metabolism in the myocardial mitochondria.

Investigation of lipid peroxidation in the myocardial mitochondria on the basis of MDA accumulation showed that panthetine, in a dose of 5 mg/kg, had a marked antioxidative action (Table 3). The MDA concentration was lowered by 57.4%. The antioxidative action of panthetine in the mitochondria correlates positively with the increase in plasma PHLA.

Investigation of the mechanism of action of panthetine by studying activities of LPL and HEL, which determine the total lipolytic activity of the plasma, showed that this substance, in a dose of 5 mg/kg, can influence LP metabolism and enhance plasma lipolytic activity.

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