ELSEVIER

European Journal of Pharmacology 351 (1998) 357–361

ejp

Effect of the calcium channel antagonist nitrendipine on lipoprotein lipase and hepatic lipase in the normal rat

Teodoro Marotta [1], Martin Bergö, Thomas Olivecrona [*]

Department of Medical Biochemistry and Biophysics, University of Umeå, Umeå, Sweden

Received 14 April 1998; accepted 17 April 1998

Abstract

Several observations indicate that a low lipoprotein lipase (LPL)/hepatic lipase (HL) ratio clusters with clinical and laboratory features of atherosclerosis. Antihypertensive treatment can unfavourably interfere with lipid metabolism, counteracting the beneficial effects of lowering blood pressure. We have evaluated the effects of the Ca^{2+} channel antagonist nitrendipine on tissue LPL and HL in the normal rat. At the dose of 40 mg/day administered intragastrically, a 5-day nitrendipine treatment induced a significant decrease in HL activity in the liver, in comparison to control animals: 656 ± 82 mU/g tissue vs. 814 ± 38 mU/g 3 h after the last administration; 640 ± 70 mU/g vs. 893 ± 101 mU/g 8 h after administration. LPL activity in heart was increased by active treatment: 2542 ± 298 vs. 2115 ± 244 mU/g in controls 3 h after administration, $P < 0.05$. At variance, LPL mass, measured 8 h after administration, was decreased in heart of treated rats: 2.38 ± 0.4 μg/g tissue vs. 3.88 ± 0.3 μg/g in controls. The ratio between heparin-releasable and residual LPL in heart was unaffected by the drug. No changes were observed in LPL activity and mass in soleus muscle or in periepididymal adipose tissue. Our results indicate that nitrendipine, at the dose used, induces changes in lipolytic enzymes of rat tissues that could be beneficial in relation to atherosclerosis. These data encourage further investigations in humans, at the usual therapeutical doses.

Keywords: Anti-hypertensive drug; Ca^{2+} channel antagonist; Nitrendipine; Atherosclerosis; Lipoprotein lipase; Hepatic lipase

1. Introduction

The role of lipoprotein lipase (LPL) and hepatic lipase (HL) in the pathophysiology of atherosclerosis has raised increasing interest in the last years (Olivecrona and Olivecrona, 1995; Beisiegel, 1996). In particular, several investigations have pointed to an association of changes in the activity of these enzymes with elements of the insulin resistance syndrome (Katzel et al., 1992; Knudsen et al., 1995; Howard, 1992; Thorn et al., 1990; Munroe et al., 1994).

Clustering of arterial hypertension and dyslipidaemia (Assmann, 1988) is a typical example of this syndrome (Reaven, 1988). Pharmacological treatment of hypertension can contribute to a derangement in lipid metabolism (Weidmann et al., 1985), partly through an interference with lipolytic enzymes. For example, β-adrenoceptor antagonists, whose untoward effects on serum lipids are known, reduce the removal rate for triglycerides from plasma, a parameter related to LPL activity (Murphy et al., 1984). On the contrary, Ca^{2+} channel antagonists are generally considered 'neutral', as far as serum lipid pattern is concerned (Kasiske et al., 1995). Favourable effects on the development of atherosclerosis have been detected during the administration of Ca^{2+} channel antagonists, in both clinical (Lichtlen et al., 1990) and experimental (Weinstein and Heider, 1989) conditions. Although not revealed by measurements of serum lipid concentrations, more subtle changes in parameters of lipid metabolism could, therefore, take place during treatment with these drugs. Together with other authors, one of us has observed an increase in the disappearance rate of exogenous triglycerides from plasma during treatment with nitrendipine, a dihydropyridinic Ca^{2+} channel antagonist (Marotta et al., 1989). The effect of this class of drugs on the triglyceride

[*] Corresponding author. Institutionen för Medicinsk Kemi och Biofysik, Umeå Universitet, S-901 87 Umeå, Sverige. Tel.: +46-90-7867725; fax: +46-90-7867840.

[1] Present address: Department of Clinical and Experimental Medicine, University of Naples 'Federico II', Naples, Italy.

0014-2999/98/$19.00

lipases involved in lipoprotein metabolism has not been studied.

The aim of this study was, therefore, to investigate whether changes in the activity and/or mass of LPL and HL in rat tissues are induced by nitrendipine treatment.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague–Dawley rats weighing about 180 g at start were purchased from Möllegaard Breeding Centre, Ejby, Denmark. The animals were kept in a light/dark cycle of 12 h, with the light period from 6 AM to 6 PM. They had free access to water and standard chow delivered by Lactamin, Stockholm, Sweden. The temperature was set at 20°C.

After an acclimation period of at least one week, nitrendipine was administered intragastrically (dissolved in polyethylene glycol, glycerol and water) at 8.00 AM for five consecutive days. The daily dose of the drug was 4 mg (17.2 ± 1.8 mg/kg) in a group of nine rats ('low dose'), and 40 mg (167.9 ± 9.9 mg/kg) in a second group of nine ('high dose'); nine animals received the vehicle only. On the fifth day of the experiment, five animals per group were killed by decapitation 3 h after administration of the drug; the remaining four rats in each group were sacrificed 8 h after the gavage. Aliquots of heart, soleus muscle, periepididymal adipose tissue, and liver were taken.

In a separate experiment, 40 mg nitrendipine was administered to 12 rats for five days at 6:00 PM; 12 rats received the vehicle. Six rats per group were deprived of food from 6:00 AM on the fifth day. At 6:00 AM the following day, rats were anaesthetised by Hypnorm® (AB Leo, Helsingborg, Sweden, 0.5 ml/kg b.wt) and diazepam (Stesolid Novum®, Dumex, Denmark, 0.5 ml/kg b.wt). Then, perfusion of coronary vessels was carried out through the aorta, by a modification of the method previously described (Liu and Olivecrona, 1991). After removal from the chest, the heart was immersed in ice-cold saline: the beat ceased immediately. The aorta was rapidly cannulated and residual blood was removed by a flush of ice-cold saline. Then, Eagle's medium oxygenated and supplemented with 5 g/dl albumin, 100 mg/dl glucose, and 14.6 mg/dl glutamine (pH = 7.4) was infused at constant velocity, keeping the pressure at about 60 mmHg. The medium temperature in the reservoir was adjusted so that the liquid flowing away from the heart reached about 35°C. Under these conditions, a regular heart beat was quickly restored (rate = 60–120 beats/min). When this was obtained, the perfusion was shifted to a medium with the same composition, but also containing 5 U/ml heparin. The perfusate was collected in ice-cold tubes for 2 min. Thereafter, the perfusion was stopped and the heart blotted dry. The ventricles were carefully cleaned from atrial and connective tissue and weighed. An aliquot was stored in the protease-inhibitor buffer (see below).

All animal procedures were approved by the Animal Ethics Committee in Umeå.

2.2. Laboratory techniques

After excision, tissue samples were immersed in a protease-inhibitor ammonia buffer and stored at −70°C until assays. The buffer contained per ml: 25 μmol NH_3, 1 mg bovine serum albumin, 10 μg leupeptin, 1 μg pepstatin, 25 kU aprotinin, 5 μmol Na_2 EDTA, 10 mg Triton X-100, 1 mg sodium dodecylsulphate, 5000 U sodium heparin. The pH was adjusted to 8.2 with HCl. For assay the samples were thawed, homogenised and centrifuged. The supernatant was immediately assayed for LPL activity as previously described (Bengtsson-Olivecrona and Olivecrona, 1992). When we centrifuged homogenised adipose tissue, three fractions appeared: a floating fat phase, an intermediate water phase, and a pellet. LPL was assayed in the intermediate phase. These data are expressed as mU/g wet tissue, where one mU is release of 1 nmol fatty acid per min. LPL mass was measured by an enzyme-linked immunoadsorbant method (Bergö et al., 1996) and is expressed as μg LPL per gram tissue wet weight.

Hepatic lipase activity in liver homogenates was measured as previously described (Bengtsson-Olivecrona and Olivecrona, 1992).

2.3. Statistical analysis

Results were analysed by two-way analysis of variance (ANOVA) and are presented as means ± standard deviation. Differences between means have been considered significant if the null hypothesis was rejected at the 5% level. When this was the case, Tukey's test for honestly significant differences or unpaired Student's *t*-test were performed for comparison between groups. The Statistical Package for Social Sciences (SPSS; Nie et al., 1975) was used for the calculations.

3. Results

The activity of HL, as measured in liver homogenates, was reduced in rats receiving high-dose nitrendipine as compared to controls, both 3 and 8 h after drug administration. This difference was statistically significant (F significance < 0.001 at the 2-way ANOVA) (Table 1).

Heart LPL was also affected by nitrendipine administration. Rats given high-dose nitrendipine had increased LPL activity in heart homogenate, as compared to controls: this difference was statistically significant 3 h after administration (Table 2). LPL mass showed a complex pattern. In controls, it was higher at 8 than at 3 h after administration of the vehicle, suggesting a circadian rhythm. This did not

Table 1
Effect of nitrendipine on hepatic lipase activity in rat liver

	Activity (mU/g tissue)	
	Hours after administration	
	3	8
C	814 ± 38	893 ± 101
L	775 ± 99	798 ± 59
H	656 ± 82[a]	640 ± 70[a]

Data are means ± S.D.
C = control group; L = low-dose group; H = high-dose group.
[a] $P < 0.05$ vs. control group.

happen in rats given nitrendipine, whose LPL mass was lower at 8 than at 3 h. This resulted in a statistically significant difference compared to controls at 8 h, and in a significant two-way drug/time interaction for LPL mass changes ($P < 0.003$).

To further explore the changes in heart LPL, we perfused hearts briefly with heparin-containing medium. This brings out a fraction of the tissue LPL, presumably corresponding to the endothelial-located 'functional' LPL. This fraction is known to be higher in hearts from fasted compared to fed rats (Borensztajn and Robinson, 1970). This effect of fasting was confirmed in the present rats, and occurred equally in the nitrendipine-treated as in the control rats (Table 3). Although not statistically significant, LPL activity was higher in both perfusion medium and remaining in the heart in the nitrendipine-treated rats, in agreement with the findings in Table 2. The important point here is that the distribution between heparin-releasable and residual LPL was the same in the nitrendipine rats as in the controls.

Lipoprotein lipase activity and mass in soleus muscle and periepididymal adipose tissue were not changed after nitrendipine treatment, in comparison with untreated animals (Table 2).

Table 2
Effect of nitrendipine on lipoprotein lipase activity and mass in rat tissues

		Activity mU/g tissue		Mass μg/g tissue	
		Hours after administration			
		3	8	3	8
Heart	C	2115 ± 244	2415 ± 238	2.75 ± 0.59	3.88 ± 0.28
	L	2226 ± 399	2286 ± 393	3.50 ± 0.40	2.66 ± 0.52[a]
	H	2542 ± 298[a]	2953 ± 519	2.72 ± 0.71	2.38 ± 0.45[a]
Soleus	C	1362 ± 99	1486 ± 178	1.81 ± 0.25	2.47 ± 0.24
	L	1411 ± 220	1502 ± 190	2.34 ± 0.65	3.27 ± 0.46
	H	1448 ± 72	1619 ± 107	2.00 ± 0.38	2.37 ± 0.33
Fat	C	1030 ± 204	1124 ± 310	3.65 ± 1.47	4.08 ± 0.53
	L	1225 ± 326	1321 ± 338	3.65 ± 0.95	3.67 ± 0.50
	H	1120 ± 439	766 ± 107	3.53 ± 1.70	3.66 ± 0.48

Data are means ± S.D.
C = control group; L = low-dose group; H = high-dose group.
[a] $P < 0.05$ vs. control group.

Table 3
Effect of nitrendipine on lipoprotein lipase activity in heparin-perfused rat heart

	Control (mU)	Nitrendipine (mU)
Perfusate		
Non-fasted	33 ± 18	44 ± 13
24 h fasted	136 ± 69[a]	158 ± 71[a]
Residual		
Non-fasted	1404 ± 227	1647 ± 462
24 h fasted	1262 ± 152	1528 ± 478

Data are means ± S.D..
[a] $P < 0.05$ vs. non-fasted animals.
These rats had received the high-dose nitrendipine and were killed 12 h after the last drug administration.

In a control experiment, we addressed the question if nitrendipine per se could affect HL or LPL activity. To resolve this matter, nitrendipine was added to homogenates of heart and liver to concentrations of 5, 10, 50, 100 and 1000 μg/ml before assay of HL and LPL activity, respectively. In a parallel experiment, the drug was added directly to the incubation medium to the same concentrations. In vitro addition of nitrendipine had no effect on HL or LPL activity.

4. Discussion

This study shows that nitrendipine decreases HL, but has relatively little effect on LPL. The doses of nitrendipine that we administered are well above the amount usually prescribed for clinical purposes (20 mg, i.e., about 0.3 mg/kg). It should be noted, however, that higher doses are necessary to reduce blood pressure in hypertensive rats than in humans: in the spontaneous hypertensive rat, for example, the dose able to reduce blood pressure by 20 mmHg (ED_{20}) is 1.2 mg/kg. In the normotensive Wistar Kyoto rat, as much as 13.9 mg nitrendipine per kg are required to obtain the same effect (Stoepel et al., 1981). Since we looked for a metabolic effect of the drug and not for its main effects (that is, the cardiovascular ones) in a normal rat, we decided to use doses in the same range of the ED_{20} in Wistar Kyoto rats and 10 times higher. Apparently, rats did not suffer from this dose of drug: their behaviour did not differ from that of control animals and their body weight at the end of the study was the same.

The administration of nitrendipine led to a dose-dependent decrease of HL activity, which reached 20–25% when the high dose was given. It is known that the drug is selectively metabolised in the liver (Meyer et al., 1983): therefore, a high drug concentration in intracellular vesicles can be expected in hepatocytes. Other pharmacological effects of nitrendipine have been shown in the liver: in particular, a cytoprotective effect against hypoxia has been observed (Thurman et al., 1988). Our finding is in agree-

ment with the observation that the release of HL is inhibited by cobalt ions (competitive Ca^{2+} inhibitors) and by verapamil, a phenylalkylaminic Ca^{2+} channel antagonist, in cultured rat hepatocytes (Rustan et al., 1986).

Lipoprotein lipase activity was not much affected by the drug. Nitrendipine binds with high affinity to cardiac membranes (Williams and Tremble, 1982). The sole tissue where we found an effect on LPL was indeed in the heart, where the activity was increased in high-dose rats, as compared to controls, without changes in the proportion of the enzyme located in the heparin-releasable pool.

While it is controversial whether high LPL levels has favourable or unfavourable effects in relation to atherosclerosis (Olivecrona and Olivecrona, 1995), experimental and clinical data indicate that an elevated HL activity is associated with conditions predisposing to accelerated atherosclerosis. The overexpression of the HL gene in the rabbit induces a decrease in high-density lipoproteins (HDLs) (Fan et al., 1994). In humans, HL activity correlates with the fractional removal rate of HDLs, while an inverse correlation between this parameter and the LPL/HL ratio is present (Brinton et al., 1991). Clinical observations confirm these physiopathologic findings. In fact, LPL/HL ratio is elevated in hyperinsulinaemic relatives of non-insulin-dependent diabetic patients (Knudsen et al., 1995). Moreover, reduced LPL and increased HL activities have been measured in hypertriglyceridaemic patients with coronary heart disease, in comparison with patients with normal triglyceride levels (Karpe et al., 1993). Thus, according to a reasonable view, a high LPL/HL ratio seems to be protective against atherosclerosis, probably because in these conditions triglycerides can be drained away from the apolipoprotein-B-containing particles and a sufficient amount of cholesterol can shift towards the HDLs. At the same time, triglycerides in HDLs are not so rapidly hydrolysed; therefore, they can be exchanged with cholesterol in very-low-density and low-density lipoproteins (Lechleitner et al., 1990).

In this light, should the changes in lipolytic enzymes that we observed during nitrendipine administration be confirmed in patients on the usual therapeutical doses, this could be regarded as a useful tool for the management of hypertensive patients.

Acknowledgements

We thank Bayer for supplying nitrendipine. The kind help of Dr. Agostino Faggiotto (Bayer Italia) in resolving practical problems concerning the handling of nitrendipine is particularly acknowledged. The useful advise by Dr. Roger Savonen and Dr. Liu Guoqing is gratefully acknowledged. We are indebted with Ms. Ann-Sofie Jakobsson and Ms. Solveig Nilsson for their kind help and highly qualified technical assistance. Mr. Sten Persson and Mr. Tord Nilsson are acknowledged for their skilled support in management of experimental animals. TM is grateful to Ms. Terry Persson for her kind welcoming and continuous, generous help. He thanks Dr. Aldo Ferrara, who encouraged and created the basis for his work in Umeå. This work was supported by the Swedish Medical Research Council (B-727) and by Bayropharm Italiana.

References

Assmann, G., 1988. The Prospective Cardiovascular Münster (PROCAM) Study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. Am. Heart J. 116, 1713–1724.

Beisiegel, U., 1996. New aspects on the role of plasma lipases in lipoprotein catabolism and atherosclerosis. Atherosclerosis 124, 1–8.

Bengtsson-Olivecrona, G., Olivecrona, T., 1992. Assay of lipoprotein lipase and hepatic lipase. In: Converse, C.A., Skinner, E.R. (Eds.), Lipoprotein Analysis. A Practical Approach. Oxford Univ. Press, Oxford, pp. 169–185.

Bergö, M., Olivecrona, G., Olivecrona, T., 1996. Forms of lipoprotein lipase in rat tissues. In adipose tissue the proportion of inactive lipase increases on fasting. Biochem. J. 313, 893–898.

Borensztajn, J., Robinson, D.S., 1970. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. J. Lipid Res. 11, 111–117.

Brinton, E.A., Eisenberg, S., Breslow, J.L., 1991. Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein–cholesterol levels with or without hypertriglyceridemia. J. Clin. Invest. 87, 536–544.

Fan, J., Wang, J., Bensadoun, A., Lauer, S.J., Dang, Q., Mahley, R.W., Taylor, J.M., 1994. Over-expression of hepatic lipase in transgenic rabbit leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. Proc. Natl. Acad. Sci. USA 91, 8724.

Howard, B.V., 1992. Obesity, lipoproteins, and heart disease. Proc. Soc. Exp. Biol. Med. 200, 202–205.

Karpe, F., Thornvall, P., Olivecrona, T., Steiner, G., Carlson, L.A., Hamsten, A., 1993. Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. Atherosclerosis 98, 33–49.

Kasiske, B.L., Ma, J.Z., Kalil, R.S.N., Louis, T.A., 1995. Effects of antihypertensive therapy on serum lipids. Ann. Intern. Med. 122, 133–141.

Katzel, L.I., Coon, P.J., Busby, M.J., Gottlieb, S.O., Krauss, R.M., Goldberg, A.P., 1992. Reduced HDL_2 cholesterol subspecies and elevated postheparin hepatic lipase activity in older men with abdominal obesity and asymptomatic myocardial ischemia. Arterioscler. Thromb. 12, 814–823.

Botnia Study Group, Knudsen, P., Eriksson, J., Lahdenperä, S., Kahri, J., Groop, L., Taskinen, M.-R., 1995. Changes of lipolytic enzymes cluster with insulin resistance syndrome. Diabetologia 38, 344–350.

Lechleitner, M., Miesenböck, G., Patsch, J.R., 1990. HDL cholesterol, triglycerides and coronary heart disease. Curr. Opin. Lipidol. 1, 330–333.

Lichtlen, P.R., Hugenholtz, P.G., Rafflenbeul, W., Hecker, H., Jost, S., Deckers, J.W., 1990. Retardation of angiographic progression of coronary heart disease by nifedipine. Lancet 335, 1109–1113.

Liu, G., Olivecrona, T., 1991. Pulse-chase study on lipoprotein lipase in perfused guinea pig hearts. Am. J. Physiol. 261, H2044–H2050.

Marotta, T., Ferrara, L.A., Pasanisi, F., Rubba, P., Iovine, C., Mancini, M., 1989. Enhancement of exogenous triglyceride removal following calcium entry blockade. Artery 16, 312–326.

Meyer, H., Scherling, D., Karl, W., 1983. Nitrendipine: identification and synthesis of main metabolites. Arzneim.-Forsch./Drug Res. 33, 1528–1534.

Munroe, P.B., Johnston, A., Duke, V.M., Daniel, H.I., Bouloux, P.M., Lawson, M., Caulfield, M.J., 1994. Investigation of lipoprotein lipase as a candidate gene for dyslipidaemic hypertension. J. Hum. Hypertension 8, 613–614.

Murphy, M.B., Sugrue, D., Trayner, I., Kaufman, S., Yashuhara, H., Dollery, C.T., Thompson, G.R., 1984. Effects of short term beta adrenoreceptor blockade on serum lipids and lipoproteins in patients with hypertension or coronary heart disease. Br. Heart J. 51, 589–594.

Nie, N.H., Hull, C.H., Jenkins, J.G., Steinbrenner, K., Bent, D.H., 1975. Statistical package for social sciences, 2nd edn. McGraw-Hill, New York.

Olivecrona, G., Olivecrona, T., 1995. Triglyceride lipases and atherosclerosis. Curr. Opin. Lipidol. 6, 291–305.

Reaven, G.M., 1988. Role of insulin resistance in human disease. Diabetes 37, 1595–1607.

Rustan, A.C., Nossen, J.Ø., Blomhoff, J.P., Drevon, C.A., 1986. Release of hepatic lipase and very low density lipoprotein by cultured rat hepatocytes. Int. J. Biochem. 18, 909–916.

Stoepel, K., Heise, A., Kazda, S., 1981. Pharmacological studies of the antihypertensive effect of nitrendipine. Arzneim.-Forsch./Drug Res. 31, 2056–2061.

Thorn, J.A., Chamberlain, J.C., Alcolado, J.C., Oka, K., Chan, L., Stocks, J., Galton, D.J., 1990. Lipoprotein and hepatic lipase gene variants in coronary atherosclerosis. Atherosclerosis 85, 55–60.

Thurman, R.G., Apel, E.D., Lemasters, J.J., 1988. Protective effect of nitrendipine against hypoxia injury in perfused livers from ethanol-treated rats. J. Cardiovasc. Pharmacol. 12 (Suppl. 4), 113–116.

Weidmann, P., Uehlinger, D.E., Gerber, A., 1985. Antihypertensive treatment and serum lipoproteins. J. Hypertens. 3, 297–306.

Weinstein, D.B., Heider, J.G., 1989. Antiatherogenic properties of calcium antagonists. Am. J. Med. 86 (Suppl. 4A), 27–32.

Williams, L.T., Tremble, P., 1982. Binding of a calcium antagonist, [^{3}H]nitrendipine, to high affinity sites in bovine aortic smooth muscle and canine cardiac membranes. J. Clin. Invest. 70, 209–212.