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ENZYME HISTOCHEMICAL EXPRESSION OF LIPOPROTEIN LIPASE IN THE LIVER AND ADRENAL GLANDS IN CLINICALLY HEALTHY RABBITS

P. Y. YONKOVA[1], P. K. ATANASSOVA[2], A. P. VODENICHAROV[1], R. S. DIMITROV[1] & P. I. HRISCHEV[3]

[1]Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [2]Department of Anatomy, Histology and Embryology; [3]Undergraduate medical student, Medical University, Plovdiv, Bulgaria

Summary

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The liver enzyme lipoprotein lipase (LPL) facilitates the release of cholesterol from lipoproteins and plays a key role in the synthesis of steroid hormones in the adrenal glands. For the current study, 6 male and 6 female clinically healthy New Zealand White rabbits, aged 3.5 months were used. Enzyme histochemical reaction (per Gomori) was performed on fresh cryostat sections, and through the Tween method, a strong positive expression of LPL in the sinusoid capillaries of the liver lobules, the lumen, and the smooth muscle cells of the walls of interlobular arteries and veins was shown. A moderate LPL expression in zona glomerulosa and weak expression in zona fasciculata were observed in the adrenal glands, whereas no LPL expression was found in both the cortical zona reticularis and the adrenal medulla.

Key words: adrenal glands, lipid metabolism, lipoprotein lipase, liver, rabbits

INTRODUCTION

The enzyme lipoprotein lipase (LPL) is produced in the adipocytes, the heart and skeletal musculature, the mammary gland's epithelial cells and the macrophages (Pentikäinen *et al*., 2002; Kojama *et al.,* 2004). Even if to a lesser extent, the liver and the adrenal glands also synthesize this enzyme (Camps *et al.,* 1991). LPL becomes physiologically active after its transfer from the tissues to the luminal surface of the vascular endothelium (Yagyu *et al.,* 2003), where it hydrolyzes the triacylglycerol-rich chylomicrons and very low-density lipoproteins (VLDL) (Markku *et al.,* 2002).

In human, rat and hamster livers, the catalytically active form of LPL is bound via heparan-sulfate proteoglycans to the hepatocytes and to the endothelium of sinusoidal capillaries, which facilitates the release of cholesterol from lipoproteins (Vilaró *et al.,* 1988; Popov, 1992; Staels *et al.,* 1996).

In guinea pigs, it was established that LPL is expressed around the lumen of the liver artery, the portal vein, in the hepato-

cytes located in the periportal areas, and in the Kupffer cells, whereas the reaction is weak around the liver veins. In the lumen of the centrilobular veins, however, no enzyme activity is detected (Camps *et al.,* 1991).

The LPL localized in the endothelium of the capillaries and the adrenal glands plays a key role in the synthesis of steroid hormones through the release of cholesterol from the lipoproteins (Gafvels *et al.,* 1991; Popov, 1992; Staels *et al.,* 1996). Furthermore, according to Staels *et al.* (1996), the function of LPL facilitates the binding of cholesterol-rich lipoprotein particles to the specific receptors on the cellular surface. Gafvels *et al.* (1991) found out that LPL-synthesizing cells could be found in all zones of the adrenal glands in rats, guinea pigs, and humans. In rats, the cells synthesizing the enzyme are found mostly in the medulla. It has been shown immunologically that LPL is also produced in the cells in the cortical middle zone, especially around lipid-filled cells. Within the adrenal medulla, intensive immunofluorescence is observed in single cells. Jansen & Birkenhager (1981) found out that in the adrenal glands of hamsters the activity of LPL was significantly lower than it was in rats.

Rabbits are a preferred animal model for studies on lipid metabolism (Araki *et al.,* 2000). In order to examine the metabolic role of LPL, Koike *et al.* (2004) generated transgenic rabbits, in which the tissue and blood plasma enzyme activity was many times higher than in non-transgenic rabbits. This increase led to a significant decrease in plasma cholesterol levels. Transgenic mice and rabbits were protected against diet-induced hyperlipidaemia (Yagyu *et al.,* 2003).

Since there were no data on LPL expression in rabbits' liver and adrenal glands in the available references, we aimed to investigate enzyme histochemical expression of LPL in the liver and adrenal glands of clinically healthy rabbits, fed a standard diet.

MATERIALS AND METHODS

Experimental animals

The experiments were performed on 12 (6 male and 6 female) clinically healthy New Zealand White rabbits, 3.5 months of age, with body weight of 3.58 ± 0.02 kg. The animals were kept in groups in cages with dimensions of 60×40×80, at ambient temperature of 20 °C, humidity of 70% and 12 hours of light per day. Each rabbit received 200 g compound pelleted feed (containing 18.3% crude protein; 12.5% crude fibres; 1.2% fats), twice a day, with constant access to water.

The euthanasia of rabbits was performed with intravenous injection of Thiopental (Biochemie, Austria), at a dose of 0.5 g per animal. The experiment was carried out after approval obtained from the Animal Ethical Committee of Trakia University, Stara Zagora

Histochemical studies

Laparotomy was performed immediately after euthanasia. Pieces of 1 cm^3 were collected from liver and adrenal glands. By means of a MTC Table Cryostat, at –20 °C, fresh cryostat sections of 6–7 μm were obtained.

Cryostat sections were processed by the method of Gomori. The enzyme histochemical reaction for LPL was based on the Tween method consisting in the deposition of insoluble calcium soaps at the sites of enzyme activity that are further converted to lead soaps and finally, in lead sulfide precipitates. On ready preparations, these precipitates appeared as

clusters of dark-brown granules (Pearce, 1960).

Analysis of data

The localization of tissue LPL expression was determined by light microscopy (Primo Star, Zeiss, Germany), and results were recorded with a digital camera Prog Res CT3 (Germany). The intensity of the reaction was assessed semi-quantitatively as per Atanassova (2000) – (0) lack of enzyme activity; (+) weak enzyme activity; (++) moderate enzyme activity and (+++) strong enzyme activity.

RESULTS

The enzyme histochemical studies revealed the presence of strong (+++) positive expression of lipoprotein lipase in the livers of all examined rabbits (Fig. 1).

The sinusoid capillaries of liver lobules (Fig. 2), the lumen of the interlobular arteries and veins (Fig. 3), as well as in the smooth muscle cells of their wall were strongly (+++) LPL-positive. A detectable reaction was also found on the lumen surface of the vascular endothelium (Fig. 3).

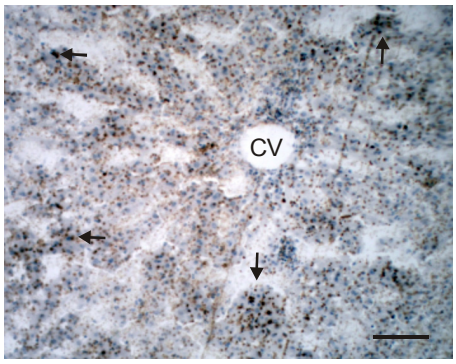

Fig. 1. Strong lipoprotein lipase histochemical expression (dark brown granules) in rabbit liver (arrows). VC – central vein. Gomori staining. Bar = 80 μm.

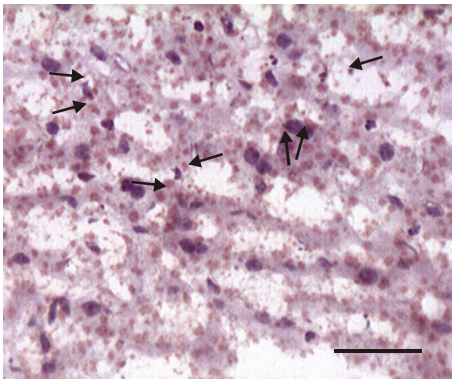

Fig. 2. Strong lipoprotein lipase histochemical expression (arrows) in sinusoid capillaries of rabbit liver. Gomori staining. Bar = 40 μm.

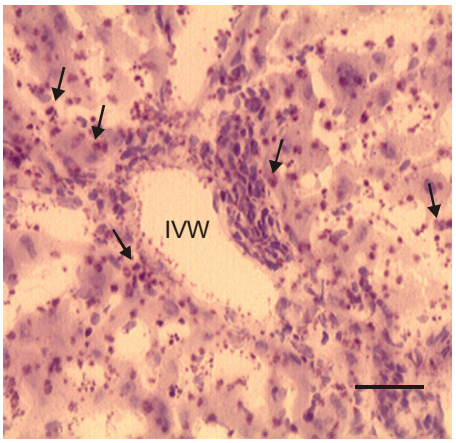

Fig. 3. Strong lipoprotein lipase histochemical expression (arrows) in the endothelium and smooth muscle cells of interlobular venous wall (IVW) in rabbit liver. Gomori staining. Bar = 40 μm.

Enzyme expression in adrenal glands, was weaker in general, compared to that observed in the liver. In the different zones of the cortex and the medulla, lipoprotein activity was very different. A moderate (++) positive reaction was observed in the subcapsular area, above the glandular parenchyma as well as around the connective tissue septa entering zona glomerulosa. A similar expression (++)

was observed in the sinusoidal capillaries around the cortical endocrinocytes of zona glomerulosa (Fig. 4).

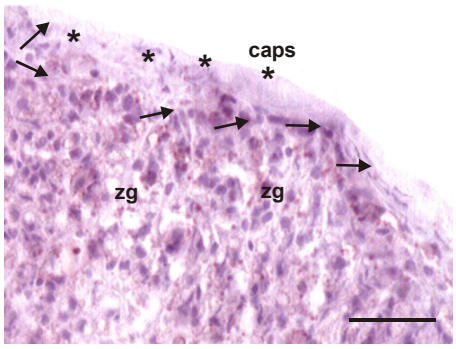

Fig. 4. Moderate lipoprotein lipase histochemical expression (arrows) in subcapsular area (*) and in zona glomerulosa (zg) of rabbit adrenal gland; caps – adrenal capsule. Gomori staining. Bar = 40 μm.

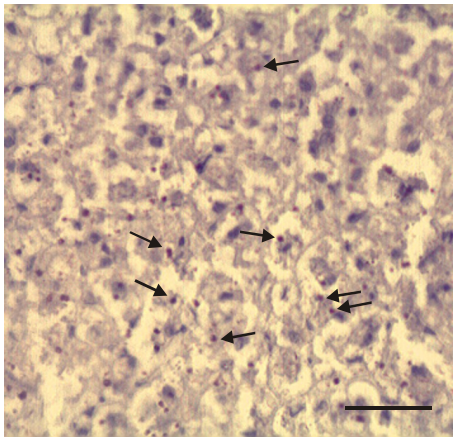

Fig. 5. Weak lipoprotein lipase histochemical expression (arrows) in zona fasciculata of rabbit adrenal gland. Gomori staining. Bar = 40 μm.

Within the cortical zona fasciculata there was a weak (+) LPL activity. Similar was the reaction in the sinusoidal capillaries in this zone (Fig. 5).

In the zona reticularis and in the adrenal medulla, no LPL enzyme expression was observed.

DISCUSSION

The light microscope observations convincingly proved that in the studied rabbits, fed a standard diet and with body weight within the breed's norms, LPL showed a strong histochemically expression in the liver and moderate to weak expression in the adrenal glands.

Similarly to Camps *et al.* (1991) who described the synthesis and enzyme expression of LPL in guinea pig's liver, we also observed a strong LPL expression in the sinusoidal capillaries, the smooth muscle cells of the walls of the interlobular arteries and veins in rabbit livers. These data correspond to what was reported by Vilaró *et al.* (1988) and Staels *et al.* (1996), according to which in humans, rats and hamsters LPL was expressed in endothelial cells of sinusoidal capillaries.

Various extents of enzyme reaction, when compared to other mammals and humans (Gafvels *et al.,* 1991), however, were observed in the adrenal glands of the examined rabbits. While the LPL-synthesizing cells in rats, guinea pigs and humans are found in all parts of the glands, in this study only a moderate LPL expression in the subcapsular area (where the respective arterial plexus was located) and in zona glomerulosa was detected. Unlike the rat (Gafvels *et al.*, 1991), in which LPL-synthesizing cells are located primarily in the medulla, in rabbits' medulla as well as in the cortical zona reticularis, no LPL reaction was found. Our data differ from the results of the abovementioned authors with regard to zona fasciculata, according to which the enzyme is synthesized in the lipid-filled cells in this zone. The reaction in our investigation was most strongly expressed in zona glomerulosa.

In conclusion, we believe that, circulating in the bloodstream, LPL makes the connection between the liver and the adrenal glands in rabbits and plays an important role in steroid synthesis.

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Correspondence:

Dr. Penka Y. Yonkova
Department of Veterinary Anatomy,
Histology and Embryology
Faculty of Veterinary Medicine,
6000 Stara Zagora, Bulgaria
e-mail: pjon@abv.bg