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Enzyme Histochemical Expression of Lipoprotein Lipase in Canine Paranal Sinus

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The purpose of the present investigation was to study the enzyme histochemical expression of lipoprotein lipase (LPS) in canine paranal sinus (PS). The results showed an intensive enzyme reaction for LPL in the cytoplasm of most cells in all layers of the stratified squamous cornified epithelium as well as in some cells of apocrine and sebaceous glands of PS. Positive LPL expression was also observed in the stroma. In the same PS structures, groups of lipid droplets of a various size have been observed. A LPL enzyme histochemical expression together with single lipid deposits of a various size was also detected in some of large blood vessels supplying PS with blood as well as in the microcirculatory bed. There was no sex dimorphism in LPL expression. The results showed that there was an intensive enzyme histological reaction in LPS in the paranal sinus that was probably related to the function of the organ, and the pathological deviations could have an impact on the intensity of reaction. This would allow the utilization of LPL enzyme histochemical expression to be used as a marker in the diagnostics of different pathological events in PS.

Key words: lipoprotein lipase, paranal sinus, dog.

Introduction

The major quantity of LPL in the body is localized in the capillary endothelium. A small amount is detected in the arterial endothelium and it is supposed to be involved in atherogenesis [16]. A subendothelial localization of the enzyme in the arterial intima is also observed. Theoretically, LPL of arterial intima could originate from circulating LPL or from the local synthesis of various cells of the intima.

Lipoprotein lipase (LPL) is the primary lipolytic enzyme, involved in the intravascular metabolism of lipoproteins [4]. This enzyme is synthesized and secreted in a catalytically active form by adipocytes and myocytes. Then, it is transported to the capillary endothelial surface. The physiological function of LPL is to hydrolyze triglycerides from chylomicrons, very low density lipoproteins (VLDL) and intermediate density lipoproteins (LDL) from the luminal side of capillary endothelium and to release free fatty acids, stored as triglycerides in the adipose tissue or oxidized for energy production in muscles [10]. The C-terminal domain of LPL is bound at a considerably higher

extent to chylomicrons and VLDL than to LDL, whereas the N-terminal domain mediates the binding to phospholipid vesicles as well as to LDL particles [7, 8]. The activity of LPL has been studied in the adipose tissue, the heart muscle, the liver, the mammary gland, and the skeletal musculature in a number of animal species and men [2, 1, 5, 3].

In the available literature, there is no detailed information about the expression and the distribution of LPL in the paranal sinus in dogs. Single reports showed traces of lipase in the apocrine cells and the secretion of sebaceous glands of this organ [11], but the type and the function of the enzyme are not still clear.

The purpose of the present investigation was to study the enzyme histochemical expression of LPL in the different structures of canine paranal sinuses and in the vessels, supplying PS with blood.

Material and Methods

In the present study, the paranal sinuses of 7 male and 7 female mongrel dogs were used.

Immediately after the euthanasia of the dogs, specimens from PS wall were obtained. The Gomori's enzyme histochemical reaction was performed on fresh cryostat cross sections for detection of positive expression of lipoprotein lipase in PS. The reaction was based upon 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, the final precipitates appeared as clusters of dark-brown granules.The lipid content was detected on cryostat cross-sections by means of histochemical reaction with Sudan III (Feinchemie KG, Sebnitz, Germany) according to Daddy. Lipid deposits were stained in yellow-orange.

Results

LPL expression was detected in the cytoplasm of most cells of all layers of the stratified cornified epithelium of the sinus. The highest number of reacted cells was observed in the basal layer and their amount decreased in the direction of stratum corneum. Clusters of brown granules with a various size were mainly observed in the apical part of glandular cells of apocrine tubules. Less frequently, enzyme activity was encountered in the basal part of secretory cells. LPL expression was established in both basal and mature cells of sebaceous glands. The expression of the enzyme was visualized as deposits of a various size in some cells of the stroma as well as in the extracellular matrix.

On serial cross-sections, we have found out that the localization of lipids in the glandular cells of apocrine tubules (Fig. 1) was the same as LPL localization in these cells (Fig. 2). Lipid deposits were also observed in the cornified epithelium, in some stromal cells, but the strongest reaction was that of sebaceous glands and their outlet duct. In the blood vessels supplying PS with blood, the pattern of LOL localization in the three vascular wall layers was irregular and appeared as single brown granules with a round shape in the subepithelial connective tissue and the endothelium of the intima, of tunica media and the adventitia. On Sudan III-stained histological cross-sections, some vessels showed single lipid deposits of a various size in the three vascular layers, reaching the intima. LPL activity was observed in the luminal surface of endothelial cells in capillaries and arterioles in the PS wall. Single reaction deposits were present in the cells of tunica media and in tunica externa of arterioles.

In this study, no sexual dimorphism in LPL and lipids expression was found out.

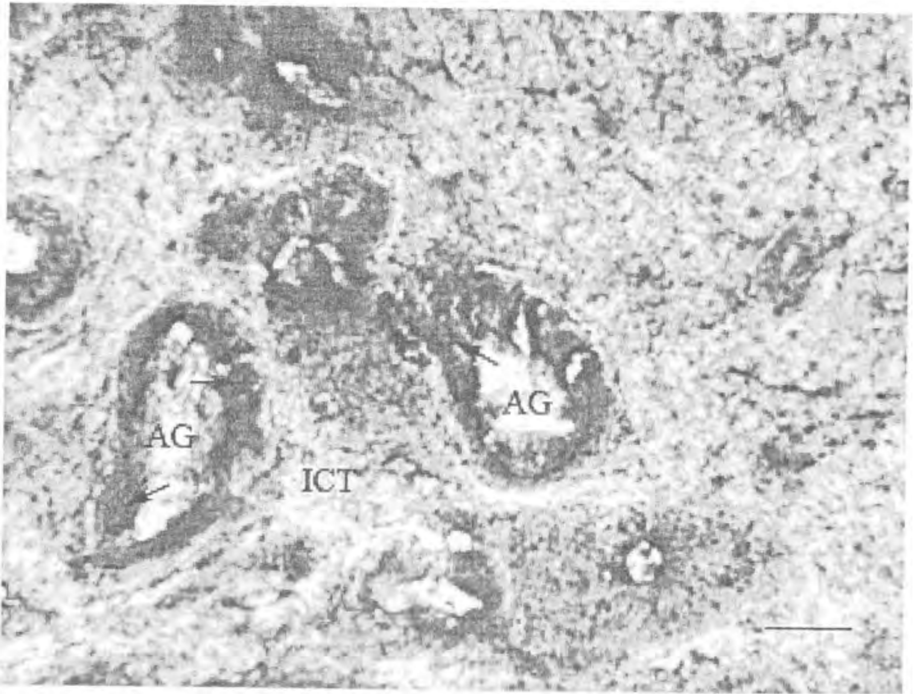

Fig. 1. Lipid droplets (arrow) in the cytoplasm of some cells of the apocrine glands (AG). ICT – interstitial connective tissue. Sudan III-H staining, × 100, Bar = 50 μm

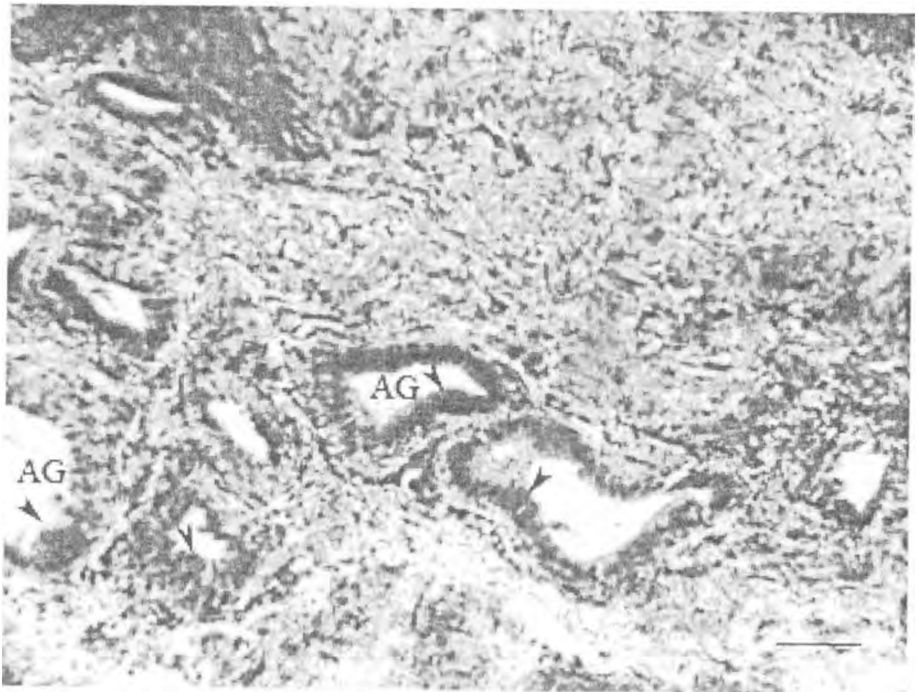

Fig. 2. Expression of LPL (arrowhead) in the cytoplasm of some cells of apocrine glands (AG), × 100, Bar = 50 μm

Discussion

The localization of LPL in PS stroma, observed by us, could be explained by the ability of this enzyme to bind to proteoglycans of the extracellular matrix, similarly to arterial intima [9]. This could result in LDL retention in these structures [9, 12]. This retention prolongs the presence of LDL in the intimal matrix that permits the modification of these particles. LPL provokes a selective uptake of cholesterol by LDL that requires cell surface proteoglycans but is not dependent on lipoprotein receptors and LPL activity [15, 13]. It is known that LPL in the arterial intima could originate from circulating LPL or its local synthesis by various cells of the intima. R. Rumsey [14] showed that LPL increased LDL binding to fibroblasts and macrophages as well as the breakdown of LDL in them. The authors established increased triglyceride concentrations in studied cells. In their view, LPL increased the uptake of lipids and lipoproteins from the cells without a LDL receptor. This pathway, in our opinion, is important for the accumulation of lipids in LPL-synthesizing cells. The data allowed assuming that the presence of lipids in the secretion of PS apocrine glands is probably related to the regulation of its strong odour.

The observed LPL localization in canine PS could be explained assuming a synthesis of this enzyme in the studied organ. On the other side, this enzyme is able to bind both to cell surface and to extracellular matrix glucosaminoglycans. This way, LPL could penetrate through the vascular wall [6, 12] and to occur in the PS stroma.

The present study provided evidence that in canine PS, there was an intensive enzyme histochemical expression of LPL that could be associated to the primary function of the gland. This expression could be utilized as a marker in the diagnostics of various pathological processes affecting the gland.

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