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Enzyme histochemical expression of lipoprotein lipase and localization of mast cells in the paranal sinus in sexually mature and immature dogs

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In this histochemical study, we established for the first time the expression of lipoprotein lipase, lipids and their relationship with mast cells localization in the canine paranal sinus. Intensive enzyme reaction for LPL was present in the cytoplasm of some cells in all layers of the stratified squamous cornified epithelium, in some cells of apocrine and sebaceous glands of PS wall in both sexually mature and immature dogs. LPL localization was also observed in the stroma.

Mast cells were observed in vicinity of the stratified squamous cornified epithelium, the apocrine and sebaceous glands of the studied organ. These structures exhibited positive reaction for both lipids in the Sudan III staining and for LPL activity. The expression of LPL, single lipid deposits of a various size and in some instances mast cells were observed in the three layers of blood vessels, supplying with blood the paranal sinus. In the microcirculation bed, LPL activity and mast cells were also shown.

In this study, there were individual particularities in LPL expression on the paranal sinus, but not such related to age or gender (sexual dimorphism).
An attempt to explain the relationship between LPL expression, lipids and mast cells localization in the PS wall was performed. This was probably related not only to the organ function, but also with the development of pathological processes within.

Key words: lipoprotein lipase, paranal sinus, dog.

Introduction

The role of mast cells in binding both lipoprotein lipase (LPL) (van Tilbeurgh et al., 1994, Kokkonen and Kovanen, 1987) and low-density lipoproteins (LDL) (Kokkonen and Kovanen, 1990) is acknowledged. This allowed some investigators to assume the involvement of these cells in the development of atherosclerosis (Ma and Kovanen, 1997). The relatively high amount of mast cells observed in canine PS

(Stefanov and Vodenicharov, 2007) is probably important not only for the normal function of the organ, but also for the development of various pathological states. Lipoprotein lipase (LPL) is the primary lipolytic enzyme, involved in the intravascular metabolism of lipoproteins (Goldberg I J, 1996). The major quantity of LPL in the body is localized in the capillary endothelium. A small amount is detected in the arterial endothelium and that part is believed to participate in atherogenesis (Zilversmit, 1973). A subendothelial localization of the enzyme in the arterial intima is reported. Theoretically, LPL of arterial intima could originate from circulating LPL or from the local synthesis of various cells of the intima. According to some authors, LPL is produced by macrophages and smooth muscle cells (Yla-Herttuala et al., 1991). This enzyme is synthesized and secreted in a catalytically active form by adipocytes and myocytes. Then, it is transported to the capillary endothelial surface. Its physiological role to hydrolyze triglycerides from chylomicrons, very low density lipoprotein (VLDL) and intermediate density lipoprotein (LDL) particles 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 (Merkel et al., 1998). LPL activity was investigated in adipose tissue in birds (Sato and Akiba, 2002) and cats (Backus et al., 2001), the heart muscle in mice (Liu et al., 2008) and sheep (Boonet et al., 2000), mammary gland in mice (Jensen, et al., 1991), canine skeletal muscle (Budohoski, 1985), rat skin (Ma and Kovanen, 1997). Montagna and Parks (1948) found out only traces of lipase in the apocrine cells and the sebum. The granules of mast cells have reacted positively. The authors did not provide detailed information about the type and mode of action of enzyme.

In the available literature, there are no data with regard to LPL expression and distribution in the paranal sinus.

The purpose of the present study was to establish the relationship between LPL activity and mast cells in canine PS.

Material and Methods

In this study, the paranal sinuses of 7 male and 7 female healthy mongrel dogs at the age of 2 months to 6 years were used.

Immediately after the euthanasia of the dogs, specimens from PS 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 is based upon the Tween method consisting in the deposition of insoluble calcium soaps at the sites of enzyme activity. They 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 and mast cells were detected on cryostat cross-sections by means of histochemical reaction with Sudan III (Feinchemie KG. Sebnitz, Germany) according to Daddy technique, replacing haematoxylin with toluidine blue with pH 3. Semi-thin cross-sections of 1 μm were stained with toluidine blue (pH 3) and Azur II.

Results

An intensive enzyme histochemical expression for LPL was observed in the cytoplasm of most cells of all layers of the stratified squamous cornified epithelium of the sinus both in adult and sexually immature dogs (Fig. 1). The highest number

of reacted cells was observed in the basal layer and their amount decreased in the direction of stratum corneum. Clusters of granules with a various size were mainly observed in the apical part of LPL-positive apocrine glandular cells (Fig. 2). 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 (Fig. 3). 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. In some dogs from both genders, LPL expression was present only in single cells of apocrine and sebaceous glands whereas others exhibited reaction in a considerable part of cells of these structures.

Mast cells were localized in the stroma, adjacently to lipid droplets, mainly near the stratified squamous cornified epithelium of the sinus, around the tubules of apocrine glands and around the acini of sebaceous glands. Less frequently, mast cells were detected among the acini in glandular complex. Single intraepithelially located mast cells (1-2 mastocytes) were observed only in some glandular tubules (Fig. 3), as well as in some acini of sebaceous glands (1-2 mastocytes).

Various-sized lipid droplets were found out at some sites in the stroma as well as in a major part of apocrine glands cells (Fig. 3). These glands exhibited a weak to moderate reaction for lipids. In some individuals, only single cells of apocrine glands were positive whereas in others, many secretory cells have reacted. In the cornified epithelium, the reaction for lipids was weak to moderate at some sites. A strong reaction was exhibited in sebaceous glands and their excretory duct, as well as in the lumen of the duct and the sinus lumen.

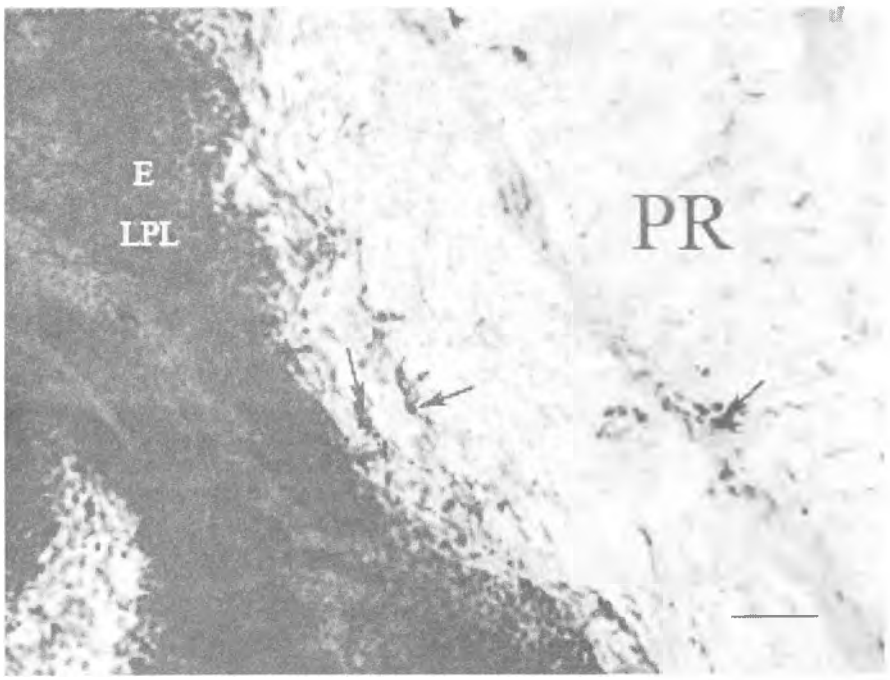

Fig. 1. Localization of (LPL) in all layers of the cornified epithelium (E), including the cornified layer. Some of cells in the propria (PR) showed a positive LPL reaction. Magnification × 100 (bar = 50 μm)

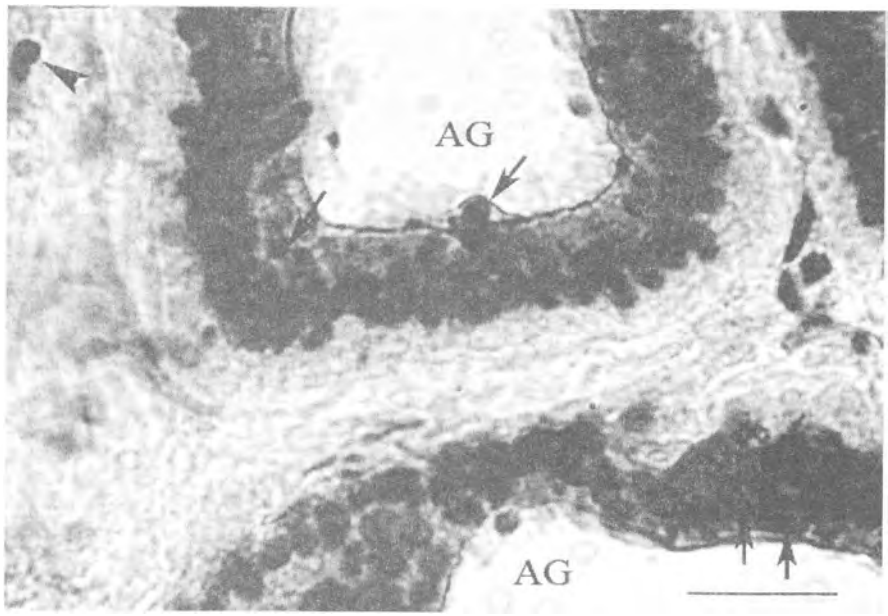

Fig. 2. Localization of LPL activity in the apical part of some secretory cells (arrows) of apocrine glands (AG), as well as in the interstitium (arrowheads). Magnification × 400 (bar = 20 μm)

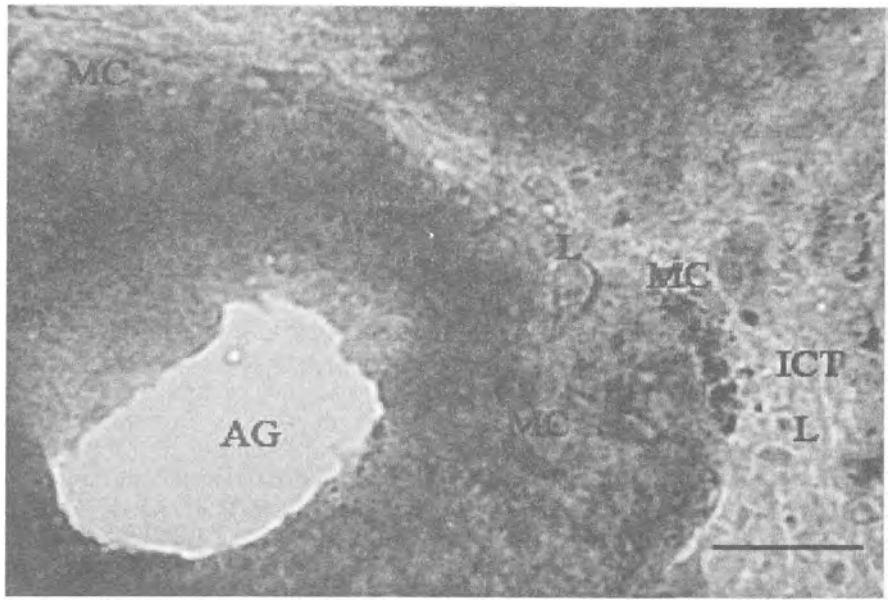

Fig. 3. Localization of lipid droplets (L) in the cytoplasm of some cells of the apocrine glands (AG) and in interstitial connective tissue (ICT). Mast cells (MC) are situated in ICT, near the apocrine glands, and single cells — intraepithelially as well. Sudan III and toluidine blue (pH 3) staining. Magnification × 400 (Bar = 20 μm)

In the blood vessels supplying PS with blood, the pattern of LPL localization in the three vascular wall layers was irregular and appeared as single brown granules with a round irregular 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. Mast cells were also detected in the three layers of some blood vessels. In the microcirculation bed, mastocytes were localized in the vicinity of capillaries, arterioles and venules. LPL activity was observed in apical part capillary endothelium. Single reaction deposits were present in the endothelium, the cells of tunica media and in tunica externa of arterioles. There were individual but neither age- nor gender-related particularities in the expression of LPL in the paranal sinus in dogs.

Discussion

The localization of LPL in PS stroma under the form of various-sized reaction deposits observed by us, could be explained by the ability of this enzyme to bind to proteoglycans of the extracellular matrix, similarly to arterial intima. This could result in LDL retention and consequently, its modification in these structures (Jonasson, 1987, Yla-Herttuala et al., 1991, Pentikainen et al., 2000). There are data that collagen-bound proteoglycan decorin could bind LPL and collagen (Pentikainen et al., 2000). The dimeric structure of LPL provokes an increased affinity to heparin, because both monomers could participate in binding one molecule heparin (van Tilbeurgh et al., 1994). Both components of mast cell granules, the proteases and the heparin proteoglycan act simultaneously to promote LDL uptake by macrophages in vitro (Kokkonen and Kovanen, 1989). The binding of LDL to mast cell granules is performed through the interaction of the apolipoprotein B component of LDL and VLDL with the heparin proteoglycan component of the granules. Secretory granules exocytosed from rat serosal mast cells bind LDL, and on being phagocytosed by macrophages, carry the bound LDL into these cells. LDL is bound to the heparin proteoglycan component of the exocytosed granules whether they are expelled into the free extracellular space or remain associated with the mast cells. The proteolytic degradation of the granule-bound LDL results in its modification such that large fused LDL particles are formed on the granule surface. phagocytosis, by macrophages, of the granules containing fused LDL particles leads to lysosomal degradation of LDL and cholesterol accumulation in macrophages as nonmembrane-bound cholesteryl ester droplets, typical of foam cells. Cholesterol is accumulated in mast cells under the form of large, partially degradated and modified LDL particles, bound to granules (Kokkonen and Kovanen, 1990). Ma and Kovanen (1997) found out that degranulation of mastocytes induced transendothelial transport and the local accumulation of LDL in rat skin. Therefore, mast cells, being an important component of canine PS stroma (Stefanov and Vodenicharov, 2008), could possess a similar activity and this could help to elucidate their role not only in lipid metabolism, but in the pathogenesis of illnesses of this organ too. The cellular and extracellular localization of lipid droplets and LPL in vicinity of mast cells, observed by us in the various structures of the paranal sinus, confirm this view of ours. The intraepithelial localization of mast cells in PS, evidenced also by using semi-thin sections, could be probably related to lipid metabolism and determined their role for physiological and pathological events in this organ. These data are corresponding to the intraepithelial localization of mast cells in the epithelium of porcine ureter, reported by Vodenicharov et al. (2005).

On the basis of data obtained in this study, we presumed a role of LPL through LPL in the pathogenesis of the frequently encountered diseases of the paranal sinus. LPL also bridges native and modified lipoproteins to heparan sulfate at the cell surface and thus facilitates the uptake of lipoproteins by intimal cells (Ory, 2007). LPL provokes a selective uptake of cholesterol from LDL — a process that requires cell surface proteoglycans but that is not dependent on lipoprotein receptors and LPL activity (Seo T et al., 2000; Pentikainen et al., 2001). On the basis of observed LPL expression in the PS wall, we hypothesize that in this organ too, the utilization of cholesterol from LDL is also possible taking into consideration of LPL ability to bind cell surface proteoglycans. The hydrolysis of VLDL by LPL results in formation of free fatty acids that increase the permeability of arteries to LDL, and consequently, causing LDL entry and retention (Rutledge et al., 1997).

In this study, the localization of LPL activity in the three layers of the vascular wall — the subepithelial connective tissue and the intimal endothelium, in tunica media and tunica externa of arteries was found out to be irregular. Single lipid deposits of a various size, and in some instances mast cells were also present in the three layers of blood vessels. In the microcirculation bed, mast cells were localized adjacently to capillaries, arterioles and venules. LPL activity was also observed in the luminal surface of the endothelium of capillaries and arterioles. Single reaction deposits were detected in the cells of tunica media and in tunica externa of arterioles. The data of the present study show that there was a relationship between mast cells, lipids and LPL (van Tilbeurgh et al., 1994; Kokkonen and Kovanen, 1990), all of them exerting their effect upon the various PS structures. LPL plays an important role in the metabolism of chylomicrons, involved in the transport of dietary lipids, as well as in the metabolism of VLDL and LDL, involved in the transport and metabolism of endogenously synthesized lipids (Smithe et al., 1998, Ory, 2007). It could be therefore assumed that exogenous lipids could influence the lipid content of sebum in canine sinus, similarly to events reported in human skin sebaceous glands (Smithe et al., 1998).

Montagna and Parks (1948) established expression of fatty acids in the apical part of tall columnar apocrine cells, in the cells and the secretion of sebaceous glands and in stratum corneum of the lining epithelium of the duct. According to them, no reaction for lipids was present in Sudan III staining as well as no cholesterol in apocrine gland cells, whereas the main amount of lipids was synthesized in the cells of sebaceous glands. Unlike these authors, we observed a positive reaction for lipids in the cells of the stratified squamous cornified epithelium, sebaceous glands, and to a lesser extent, in apocrine glands cells and the stroma in Sudan III staining. It is proved that the skin and skin glands were important sites for de novo lipid synthesis in primates and rats (Feingold et al., 1982, 1983). It is known that apocrine and sebaceous glands in human skin dermis secrete lipids, mainly triglycerides, fatty acids, cholesterol and its esters. Low density lipoprotein receptor (LDL R) and LPL were also detected in skin apocrine and sebaceous glands. These data demonstrate that these glands could release endogenous cholesterol and fatty acids and that this could be important for understanding both acne and axillary odour (Smithe et al., 1998). By means of the observed LPL localization in the cells of apocrine, sebaceous glands and the lining epithelium of PS, we support the opinion of some investigators, affirming that in most cells, cholesterol is probably synthesized de novo or endogenous cholesterol under the form of LDL is uptaken by LPL and glycosaminoglycans (Smithe et al., 1998; Ory, 2007). Via regulating the metabolism of lipoproteins, LPL enhances the extracellular accumulation of lipids (Pentikainen et al., 2001). We share a similar opinion, assuming that this pathway is important for

lipid accumulation in LPL-synthesizing cells. Our data allowed believing that the presence of lipids in the secretion of PS apocrine glands was probably related to the control of its strong odour.

It could be speculated that the observed localization of LPL in canine PS could be a result from synthesis in the studied organ. Furthermore, LPL is able to bind to glycosaminoglycans both on cell surface and of the extracellular matrix. This way, LPL could penetrate through the vascular wall (Jonasson, 1987; Yla-Herttuala et al., 1991; Pentikainen et al., 2000) and to occur in PS structures.

The present investigation provided evidence that in the paranal sinus, there was an intensive enzyme histochemical expression of LPL and lipids i both sexually mature and immature dogs that could be attributed to the main function of the organ. Taking into consideration the results of our study, a possible relationship between LPL and lipids expression, on one hand, and the localization of mast cells in PS, on the other, could be assumed. This was probably important not only for the course of physiological, but for the pathological events in this organ.

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