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Blood Supply of Canine Paranal Sinus

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The investigations on macro- and microvascularization of the canine paranal sinus showed that the main vessels supplying it with blood were the dorsal perineal artery (from the caudal gluteal artery) and the caudal rectal artery – from the ventral perineal artery (a branch of the internal pudendal artery). From them, 1 or 2 Aa. sinus paranalis are separated, that supply with blood the wall of the sinus. On their entry into the wall, they divide into branches that supply with blood the apocrine and sebaceous glands. These branches give rise to arterioles and capillaries forming a dense blood vessels network. The morphometric study showed that the size of arterioles and venules in the connective tissue between the basal membrane of the stratified squamous cornified epithelium and apocrine glands was smaller than that of arterioles and venules of connective tissue between apocrine glands and anal sphincters.

Key words: vascularization, sinus paranalis, dog.

Introduction

By reason of the clinical importance of the paranal sinus in dogs [14] this organ has been subject to numerous histological and histochemical investigations [3, 7, 8, 9]. The information about the vascularization of canine paranal sinus is relatively scarce. According to Baker [1], the arteries supplying blood to the canine paranal sinus are the perineal artery, the caudal haemorrhoidal artery, branches of the internal pudendal artery and the caudal gluteal artery (the caudal branch of the internal iliac artery). These arteries are accompanied by the respective satellite veins. Gerisch and Neurand [5] demonstrated that in dogs, many capillaries were encountered in the vicinity of the sinus glands. From comparative point of view, the blood supply of anal sacs in cats are studied in detail. Godynicki et al. [6] reported that feline anal sac received blood from the ventral perineal artery and the caudal rectal artery, branches of the internal pudendal artery. In the view of the authors, the caudal rectal artery diverged in the anal sac wall and supplied it with blood, whereas the excretory duct of the anal sac receives blood mainly from the ventral perineal artery. Anal sac arteries ramify in its wall, giving rise to branches for apocrine glands (Rr. glandulae apocrine), sebaceous glands (Rr. glandulae holocrine) and the epithelium (Rr. epitheliales). These branches form small capillary networks. The venous drainage is performed by veins, oriented parallelly to arteries.

The scarce information about the blood supply of the paranal sinus in dogs motivated the present investigation aimed to clarify the macro- and microcirculation of the organ.

Material and Methods

Immediately after euthanasia of dogs with 5% Thiopental solution (Biochemie, Austria) i.v., the blood vessels of the pelvic cavity were washed with saline. For visualization, 5% solution of Indian ink-gelatin (37 °C) was injected [11] in the internal iliac artery of three male and two female mixed breed dogs at the age of 3-6 years.

Micrometric investigations were performed on permanent histological preparations stained with Erlich eosin made from material obtained from the paranal sinus of 8 healthy mixed-breed dogs euthanized with 5%.Thiopental solution (Biochemie, Austria) i.v. and fixed in 10% formalin. The sized of blood vessels in the paranal sinus wall was measured by means of light microscope ZEISS Primo Star, Germany, camera Progres, Capture 2.6 – JENOPTIK Laser, Optic, and analysis programme of Soft Imaging Sistem GmbH.

Investigation of the microcirculatory bed

The blood vessels from the microcirculatory bed in the paranal sinus wall were visualized by the reaction of Sherer-Singler et al. [10], used to detect the enzyme histochemical expression of endothelial NADPH-diaphorase.

Immediately after the euthanasia, pieces of 1 cm^3 were obtained from different parts of the organ and put immediately in 4% paraformaldehyde (Sigma Aldrich Chemie, Switzerland) in phosphate-buffered saline (PBS), pH 6.9, for 24 h at 4°C. Cross-sections of 10–20 μm were prepared on a freezing microtome (Slee, Mainz, Germany) and then, they were processed by the technique of Sherer-Singler et al. (1983) as free-floating sections by incubation in a solution containing nitro blue tetrazolium (0.2 mg/ml, Sigma Aldrich Chemie GmbH, Germany), β-NADPH (Santa Cruz Biotech, Santa Cruz, CA, USA) Triton X-100 (0.5%) (Merck Belgalabo, Overisje, Belgium) in PBS (0.1 M, pH 7.4) for 1-2 h at 37°C. This technique was chosen as the Indian ink-gelatin contrasting did not give a satisfactory result.

Cryostat cross-sections of 6–7 μm were prepared from the same areas. They were slide-mounted, fixed for 1 h in Carnoy's fixative, dehydrated in ascending alcohol series and stained with 0.1% solution of toluidine blue in McIlvane's buffer, pH 3 [12].

Histochemical detection of collagen and elastin fibres in blood vessels' wall

Material from the paranal sinus wall was fixed in 10% neutral formalin, dehydrated in alcohol series, cleared in xylene and embedded in paraffin. Paraffin cross sections of 5–7 μm were stained with Elastica Van Gieson Staining Kit (MERK, Germany); collagen fibres were stained in red and elastic fibres – in black.

Data were statistically processed by the *t*-test, StatMost for Windows, at a level of significance $P<0.05$.

Results

Using carcasses filled in situ with Indian ink-gelatin through the iliac artery, we demonstrated that the vascularization of the canine paranal sinus was performed by the dorsal perineal artery, a branch of the caudal gluteal artery and by the caudal rectal

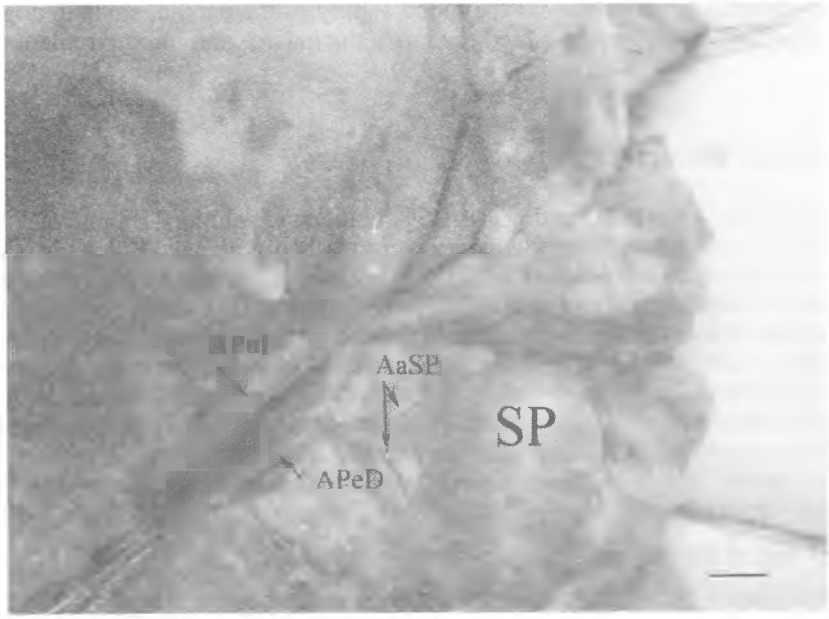

Fig. 1. Both Aa. Sinus Paranalis (ASP) Separate with a Common Trunk from A. Perinealis Dorsalis (ApeD), a Branch of A. Glutea Caudalis. Apul – A. Pudenda Interna ; SP – Sinus Paranalis. Blood Vessels Filled with Indian Ink-Gelatin. Bar=1cm

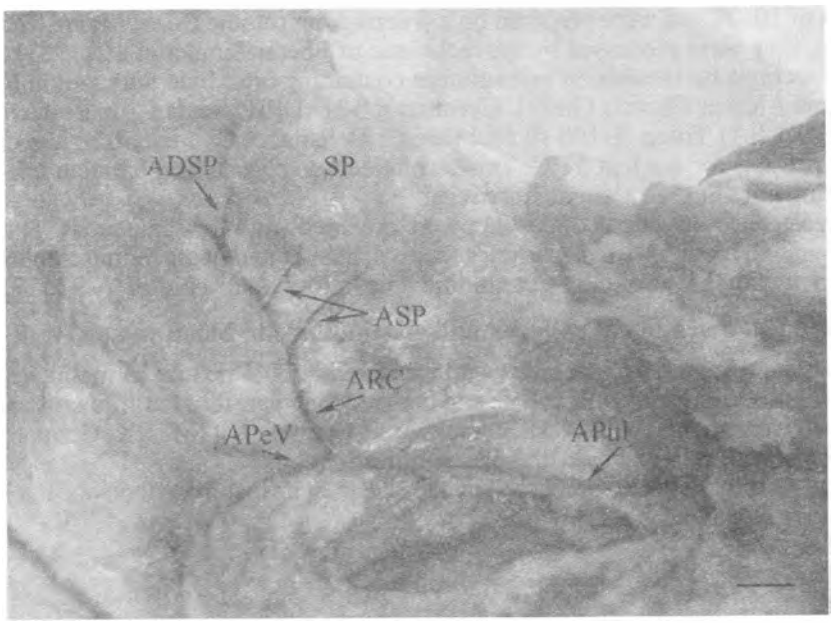

Fig. 2. Both Aa. Sinus Paranalis (ASP) Separate Individually from A. Rectalis Caudalis (ARC), a Branch of A. Perinealis Ventralis (APeV) (Branch of A. Pudenda Interna: APul). A. Ductus Sinus Paranalis (ADSP) also Separates from ARC. SP – Sinus Paranalis. Blood Vessels Filled with Indian Ink-Gelatin. Bar=1cm

artery – branch of the ventral perineal artery (branch of the internal pudendal artery). In the different subjects, the dorsal perineal artery ramifies in 1 or 2 branches near to the paranal sinus, which represent Aa. sinus paranalis (Fig. 1). One or two anal sac arteries also diverged from the caudal rectal artery, either independently or from a common trunk (Fig. 2). After reaching the sinuses, Aa. sinus paranalis give branches for the external anal sphincter, the apocrine and holocrine glands, and further ramify in the sub epithelial connective tissue, forming a dense network in the sinus wall. The branches for apocrine glands formed a glandular vascular network, some vessels from which penetrated among the acini and formed a periacinar vascular network. The veins accompany the respective arteries and joined the veins of the sinus. The blood supply of paranal sinus apocrine glands was done by branches for apocrine glands, originating from the arteries of the sinus. Adjacently to tubules, they continued into a periglandular capillary network, from where the blood passed into a peritubular capillary network, visualized by both toluidine blue staining and by detection of a moderate expression of NADPH-d activity in the vascular endothelium. The branches directed onto the basal membrane of the epithelium formed a subepithelial capillary network.

The light microscopy of microcirculatory bed vessels showed that the tunica interna of arterioles was composed of an endothelial layer, a thin subendothelial layer and an inner elastic membrane. This membrane was absent or only some segments of it were preserved in the smallest arterioles of a size < 12 μm. The tunica media contained 1-3 layers of smooth muscle cells. The external elastic membrane was absent. The tunica externa consisted of loose connective tissue where collagen fibres prevailed. The size of arterioles (33.9 ± 14.1 μm, mean ± SD) in the subglandular connective tissue (SGC) and in the interstitial tissue (IS) between the tubular apocrine glands was statistically significantly bigger than that of arterioles (12.8 ± 4.5 μm, mean ± SD) in the subepithelial connective tissue (SEC). Arterioles arborized into dense capillary networks. The wall of capillaries was represented by the tunica interna, that contained an endothelial layer with 1-3 endothelial cells, basal lamina and pericytes. The size of venules was also variable: from 13.7± 2.9 μm (mean ± SD) in the subepithelial connective tissue to 53.6 ± 19.2 μm (mean ± SD) in the subglandular connective tissue. Postcapillary venules had a structure similar to that of capillaries, but with larger lumens. They passed into venules of a larger size with a continuous pericyte layer. With increase in venules' size, a smooth muscle cell layer (muscle venules) was formed. Although thin, the tunica externa was also observed.

The excretory duct of the paranal sinus in this investigation was found to be vascularized by A. ductus sinus paranalis, originating from the caudal rectal artery (Fig. 2). The size of arterioles and venules in the excretory duct stroma also decreased towards the stratified squamous cornified epithelium.

Discussion

The vascularization of canine paranal sinus through the dorsal perineal artery, observed in this study, was similar to data reported by Baker [1]. In our investigation, however, we describe for the first time the blood vessels that enter and ramify into the wall of the sinus. These vessels were called by us Aa. sinus paranalis by analogy to the vessels in cats illustrated by Godynicki et al. [6]. With regard to the way Aa. sinus paranalis separate, our results support the findings of Godynicki et al. [6] that feline sinus arteries were more numerous (2-5). The data from our observations on intraorgan vessels and microcirculatory bed allowed us to assume that the vascularization in the dog was similar to that described by Godynicki et al. [6] in the cat. The names of branches as

suggested by these authors, could be also used for the respective branches in dogs. For example, after reaching the sinuses, Aa. sinus paranalis give rise to branches for the external anal sphincter muscle, for apocrine glandular tubules and holocrine glands, termed as Rr. glandulae apocrinae and Rami glandulae holocrinae in the cat, and then arborize in the subepithelial connective tissue (R. mucosae) and form a blood vessels network in the sinus wall. Rami glandulae holocrinae form a periglandular blood vessel network, from where blood vessels penetrate among the acini to form a periacinar capillary network. Venous vessels accompany the respective arterioles and join the sinus veins. The blood supply of apocrine sinus glands is performed by Rami glandulae apocrinae, originating from the sinus arteries or from Rami glandulae holocrinae [6]. In the vicinity of tubules, they go on in a periglandular vascular network that was visualized by toluidine blue staining and by the moderate degree of NADPH-d expression in the vascular endothelium. The expression of NADPH-d activity in paranal sinus vascular endothelium assisted for their visualization and also, provided evidence for confirming the conclusion of Bull et al. [2] about the influence of nitric oxide on skin blood vessels. By the measurements of the size of blood vessels from the microcirculatory bed, we add to the information of Godynicki et al. [6] about the vascularization of feline paranal sinus. The size of capillaries in the subglandular, interstitial and subepithelial connective tissue varied from 2.9 to 8.1 μm [4]. The size of arterioles and venules was statistically significantly bigger in the subglandular and interstitial connective tissue as compared to the size of these vessels in the subepithelial connective tissue ($p<0.001$). The Van Gieson staining showed that the inner elastic membrane was absent in arterioles of a size under 12 μm or only some segments were preserved. This confirmed the findings of Dellmann and Eurell [4], about the disappearance of the inner membrane or presence of some segments only from the smallest arterioles.

The excretory duct of the canine paranal sinus was vascularized by A. ductus sinus paranalis, originating from the caudal rectal artery – branch of the ventral perineal artery, dissimilar to cats where the A. ductus sinus paranalis comes from the ventral perineal artery [6]. The size of arterioles and venules in the excretory duct stroma also found to decrease towards the stratified squamous cornified epithelium , thus adding to the available information about canine paranal sinus vascularization.

In conclusion, this study showed that the wall of the paranal sinus, including its excretory duct in dogs was richly vascularized, determining an enhanced production of secretion from apocrine glandular tubules and holocrine acini.

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