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Morphological and neuroanatomical study of the mammary gland in the immature and mature European beaver (*Castor fiber*)

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ABSTRACT

This study investigated general morphology and immunohistochemical properties of nerve fibres supplying the mammary gland (MG) in the European beaver. The microscopic analysis of the beaver mammary gland revealed the presence of morphological structures which are characteristic for mammals. There were no distinct differences in the morphological features of the mammary gland between the juvenile and non-pregnant mature beaver.

The nerve fibres were visualized using antibodies against protein gene product 9.5 (PGP) and biologically active substances including β -hydroxylase tyrosine (D β H), neuropeptide Y (NPY), calcitonine gene-related peptide (CGRP) and substance P (SP). The study has revealed that the MG in the juvenile and mature beaver is richly supplied with PGP-immunoreactive (PGP-IR) nerve fibres. The most abundant innervation was observed in the nipple and less numerous nerve terminals supplied the glandular tissue. Double-labelling immunohistochemistry disclosed that the majority of PGP-IR nerve fibres associated with blood vessels and smooth muscle cells in both the nipple and glandular tissue were also D β H-IR. However, these nerve terminals were less numerous in the glandular tissue than in the nipple. Most of the D β H-IR axons associated with arteries and smooth muscle cells in the entire gland also stained for NPY. Small number of D β H/NPY-IR fibres supplied veins. CGRP-IR fibres were more abundant than those expressing SP. No distinct differences in the distribution and immunohistochemical characteristic of nerve fibres were observed between the juvenile and adult animals. The distribution and immunohistochemical properties of nerve fibres supplying the gland in the beaver remind those previously described in other mammalian species.

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1. Introduction

The European beaver (*Castor fiber*) is the largest rodent of Eurasia. The body weight of these animals reaches 18–25 kg, sometimes exceeding 30 kg. Beavers are strongly territorial animals. They mark occupied territory by castoreum which gives important information about the composition of the family, gender and social hierarchy. Beavers are monogamous animals. Female beavers generally get pregnant in January and February. The newborns are born in May and June after 105–107 days of pregnancy. In the first month of life beavers eat only nutrient-rich, high fat milk. The females give

birth once a year, in which there are one or two, rarely four young (Czech 2004; Dzięciołowski, 2004). European beavers live to about 30 years, but the period of their intensive breeding falls between 5 and 10 years of age. They live in permanent families. The typical family consists of a parental pair, newborns and the juveniles from the previous year. After one year of life, the weight of the juveniles is 8–13 kg, after the second year reaches up to 14 kg and the three-year and older animals weigh up to 21 kg. The second generation at the age of 2.5 years leaves the family nest (end of summer) and as a sexually mature adult starts to find a sexual partner (Czech, 2004).

The beaver is one of these animals that rarely influence the formation of the ecosystem (Dzięciołowski, 2004; Rosell et al., 2005; Kukuła et al., 2008; Campbell-Palmer and Rosell, 2015) therefore, it has become the subject of scientific observations and research. However, its organs are unique, because the beaver is found on the list of species under special protection in the European Union.

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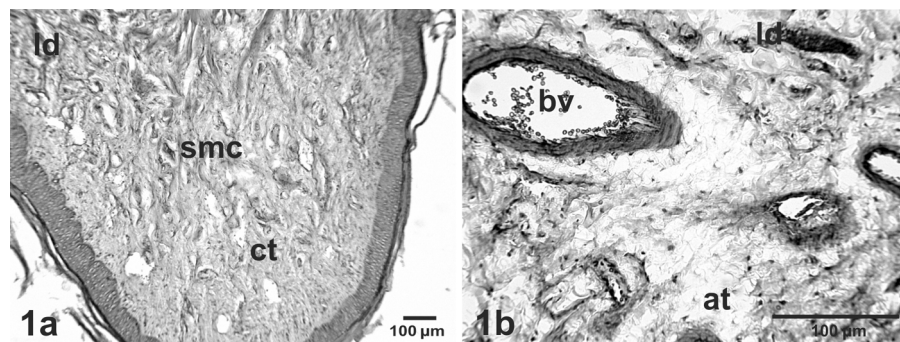


Fig. 1. The section of the nipple (a) and parenchyma (b) of the juvenile beaver mammary gland. Hemotoxylin-eosin staining. 1a—connective tissue (ct), smooth muscle cells (smc) and developing lactiferous ducts (ld) in the nipple. 1b—cross section of the blood vessel (bv), interlobar ducts (ld) and adipose tissue (at) in the parenchyma.

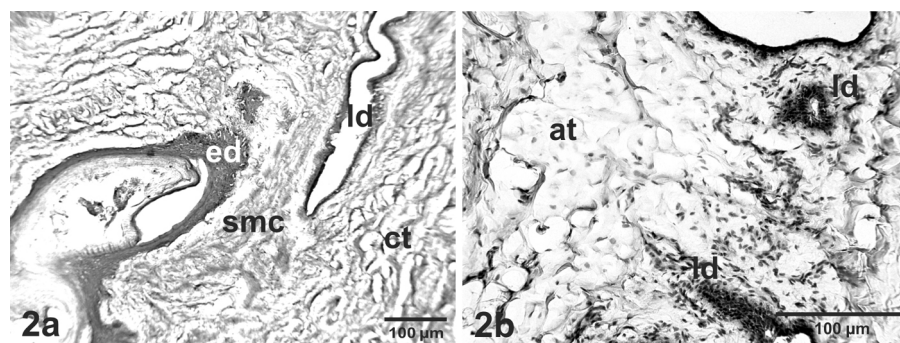


Fig. 2. The section from the nipple (a) and parenchyma (b) of the mature beaver mammary gland. Hemotoxylin-eosin staining. 2a—connective tissue (ct), smooth muscle cells (smc) and lactiferous ducts (ld) in the nipple. 2b—interlobar ducts (ld) and adipose tissue (at) in the parenchyma.

According to the Polish law, the beaver is a species partially protected. Therefore, in the areas where the animals are in conflict with the human interest (overpopulation) it is possible to get permission to hunt them given by the Regional Directorate of Environmental Protection. This makes the opportunity to collect the organs to perform investigations which augment the knowledge on the beaver biology.

The main directions of these studies were the assessment of the population, distribution, behavior, biology, or genetics (Lavrov, 1979; Doboszyńska and Żurowski, 1983; Brzuski and Lubowiecka-Kulczyka, 1999; Goodman et al., 2012; Halley et al., 2012) but still very little is known about the morphology of beaver organs. So far the major attention has been paid to the reproductive system. Previous and general observation dealing with the reproductive tract appeared in the 50 s of the twentieth century (Hinze, 1950). Later, research was focused on the morphology and physiology of the female (Brenner, 1964; Provost, 1962; Larson, 1967; Gienc and Doboszyńska, 1972; Doboszyńska, 1978) and male (Żurowski, 1977; Doboszyńska and Żurowski, 1983) reproductive organs. These studies provided valuable data dealing with the biology of the reproductive processes (Doboszyńska, 1978; Doboszyńska and Żurowski, 1981). Earlier studies have also investigated the morphology of beaver anal glands (Walro and Svendsen, 1982; Svendsen, 1978), kidneys, cardiac muscles, lungs (Dolka et al., 2014) or testicles (Bierla et al., 2007). Recent contributions have provided some interesting detailed information on the structure and function of other beaver organs. For instance, the beaver digestive system has several unique morphological features including the cardiogastic gland found near the oesophageal entrance (Ziółkowska et al., 2014). Another intriguing issue deals with the spleen which represents a defensive rather than a storage type (Dolka et al., 2014). These interesting data encourage the expansion of research of this species.

Although much information has been gained about the anatomy of the beaver reproductive tract, there is still no data dealing with the morphology of the mammary gland in this species. Furthermore, there is also no information on the innervation of some organs including the mammary gland in the beaver. Earlier studies have revealed that nerve fibres supplying the rat (Eriksson et al., 1996) or pig (Franke-Radowiecka, 2011; Franke-Radowiecka et al., 2015) mammary gland derive from dorsal root ganglia (DRG) and sympathetic chain ganglia (SchG). Therefore, the aim of the present study was to investigate the expression and coexistence of biologically active substances, commonly considered as markers of sympathetic [β -hydroxylase tyrosine (D β H), neuropeptide Y (NPY)] or sensory [calcitonine gene-related peptide (CGRP), substance P (SP)] nerve structures, in nerve terminals supplying the beaver mammary gland using immunohistochemical methods. The nerve fibres were visualized using antibodies against panneuronal marker protein gene product 9.5 (PGP 9.5).

2. Materials and methods

Sexually immature (n=3, aged about 1 year, body weight – n1=8.1; n2=10.4; n3=11) and mature (n=4, aged about 3 years, body weight–n1=17.1; n2=16.6; n3=21; n4=18.7) female European beavers were used. The animals were caught using nets by a specialized team of the Polish Hunter Association from Suwalki (Poland). The procedures applied were based on the permission from the National Ethics Commission for Animal Experimentation, Polish Ministry of Science and Higher Education, and from the Regional Directorate for Environmental Protection in Olsztyn (Poland), a government institution responsible for wildlife management in the Warmia and Mazury Voivodship of Poland. The animals were deeply anaesthetized with xylazine (0.1 ml/kg body weight, i.m., Sedazin, Biowet Puławy, Poland) and ketamine (0.1 ml/kg body

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