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# NADPH-d expression in mast cells of porcine tube auditivae

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The aim of the present study was to determine the expression of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) in mast cells of tuba auditiva (Eustachian tube) in domestic pigs. NADPH-d positive cells were observed in the propria of the cranial, middle and caudal parts of tube's cartilaginous portion. They were located mainly adjacently to blood vessels, mucosal glands and epithelium. The finding that mast cells of porcine tube Eustachian could synthesize nitric oxide could throw more light on the regulation of physiological events within the organ.

The presence of NADPH-d positive mast cells in the Eustachian tube wall in pigs confirms their ability to synthesized nitric oxide.

*Key words:* mast cells, NADPH-d, tuba auditiva, pig.

## Introduction

A number of researchers have investigated the distribution of mast cells in the Eustachian tube in different animal species and humans, both under normal and allergic conditions [11, 5]. The localization of NADPH-d positive mast cells in the pig kidney is described in detail by Vodenicharov and Bozhilova-Pastirova [10]. No data are available about the ability of Eustachian tube mast cells for nitric oxide synthesis. Nicotinamide adenine dinucleotide phosphate-diaphorase is an important element of the metabolic pathway of nitric oxide (NO) synthesis [9]. The role of endogenously produced NO in the neurotransmission, smooth muscle relaxation and immune response is acknowledged [2]. The lack of data about the presence of nitric oxide synthesis in porcine Eustachian tube was the reason for performing the present study.

The purpose of the investigation was to reveal the expression of NADPH-d in Eustachian tube mast cells in the pig.

## Material and Methods

From different areas of the pharyngeal part of the Eustachian tubes of 6 male and 6 female healthy pigs (Landrace × Bulgarian White crosses), pieces of 1 $cm^3$ were collected.

They were immediately put in 4% paraformaldehyde (Sigma Aldrich Chemie, Switzerland) in phosphate-buffered saline (PBS), pH 6.9 at 4°C and after being fixed, washed with 0.01M PBS with pH 7.2. On a freezing microtome (Mainz, Germany) 10–20 μm cut sections thickness were prepared. Further, the free-floating sections were processed by the NAPDPH-d histochemical technique of Sherer-Singler et al. [8] by incubation in a solution containing 0.2 mg/ml nitro blue tetrazolium (NBT) ( Sigma Aldrich Chemie GmbH, Germany), 1mg/ml β-NADPH (Sigma Aldrich Chemie, Switzerland) and 0.5% Triton X-100 (Merck Belgalabo, Overisje, Belgium) for 1 h at 37 °C. After the development of the colour, two washings were performed: first in 0.1M Tris HCl and second, in 0.01M PBS.

From the same specimens, fixed in Carnoy's fixative, 6 μm cut sections were prepared. They were stained with 0.1% solution of toluidine blue in McIlvane's buffer, pH 3 [6].

## Results

In this study, NADPH-d positive cells were detected in the cranial, middle and caudal parts of the Eustachian tube's cartilaginous portion. The reaction was observed in the granules of these cells, but not in their nuclei. NADPH-d positive cells were localized mainly in the subepithelial connective tissues (Fig. 1). Some of them were observed

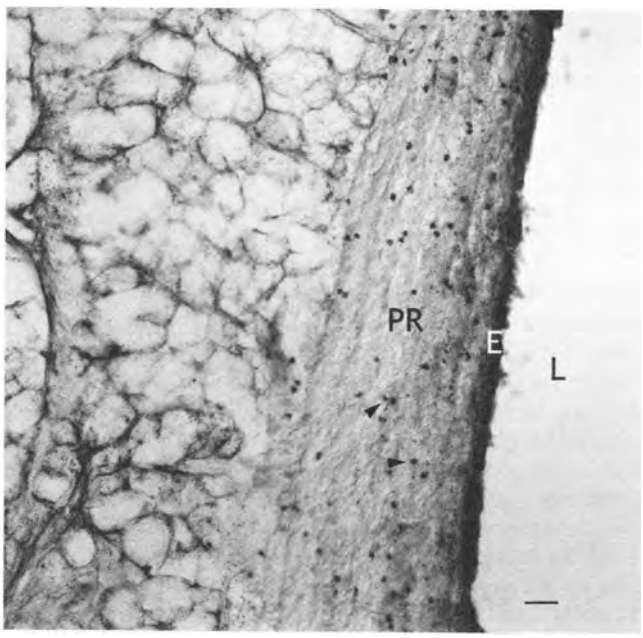


Fig. 1. NADPH-d positive cells (arrowheads) localized in the propria (PR). E - epithelium of the organ, L - lumen. Bar = 60 μm.

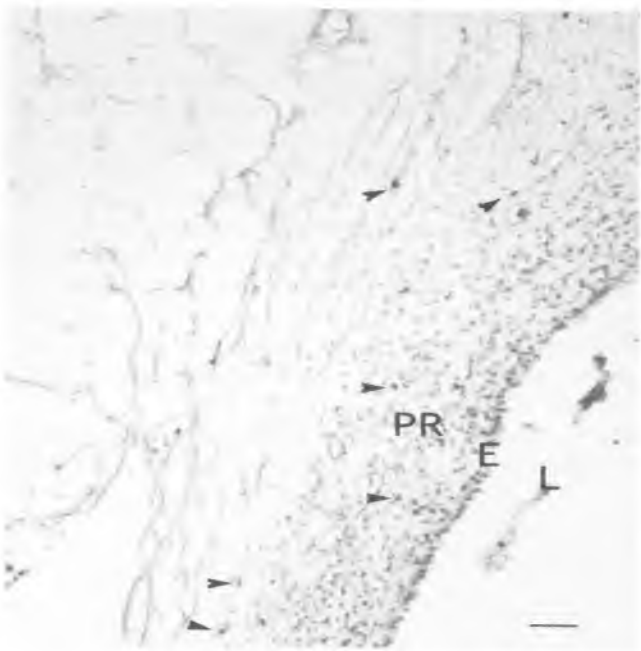


Fig. 2. Mast cells (arrowheads) stained with toluidine blue showed the same localization as the NADPH-d positive cells. PR - propria, E - epithelium of the organ, L - lumen. Bar = 60 μm.

in the vicinity of cells of microcirculatory vascular bed, gathered in clusters. Single NADPH-d positive cells were situated around the glands. The toluidine blue staining of cut sections of the same specimens showed a similar localization of NADPH-d positive cells and mast cells (Fig. 2).

## Discussion

Enzyme histochemistry and subsequent toluidine blue staining demonstrated that NADPH-d positive cells were probably mast cells. The finding that mast cells in the wall of this organ possessed NADPH-d activity agrees with the research data of Vodenicharov and Bozhilova-Pastirova [10] and confirms that mast cells are a site of nitric oxide synthesis. The location of mastocytes near the blood vessels was probably related to the smooth muscle relaxation, due to released nitric oxide [1]. On the other hand, this localization of mast cells could be attributed to their ability to synthesize and release a number of inflammatory mediators [3]. The role of nitric oxide in mucin synthesis by the glands in the middle ear is acknowledged [7]. Based on cited data, we suggest that nitric oxide released by mast cells could probably influence the secretion of glands in the Eustachian tube in the pig as well. Different researchers have studied the localization and distribution of mast cells in the Eustachian tube and the middle ear of guinea pigs and humans and observed increased counts in allergic states [11, 5]. It could be therefore hypothesized that mast cells mediators are involved in similar pathological events in porcine Eustachian tube.

## Conclusion

The present study revealed that mast cells, located in the wall of the pharyngeal part of the Eustachian tube in healthy pigs, could synthesize nitric oxide, which could play an important role in the functioning of the organ.

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