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Neuronal nitric oxide synthase (NOS I) in the buffalo epididymis

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ABSTRACT: The localization of neuronal nitric oxide (NOS I) in the buffalo epididymis have been investigated by nicotinamide adenite dinucleotide phosphatase-diaphorase (NADPH-d) histochemichemistry to the light microscope (LM) and NOS immunoistochemistry to the scanning electron microscope (SEM), respectively.
Histochemistry: examination of epididymis specimens revealed an intense NADPH-d staining in the basal cell epithelium and endothelium cells of blood vessel. The NADPH diaphorase staining was diffuse and granular only along the caput epididymal epithelium. NADPH diaphorase staining was less intense or absent in the corpus and in the cauda of epididymis. Dense NADPH diaphorase is labeling in the endothelium of blood vessels along the whole buffalo epididymis.
Immunoistochemistry: intense NOS I immunoreactivity was detected in the caput epididymis specimen by immuno-SEM. The basal epithelium showed intense and wide-spread immunoreactivity. In the corpus and in the cauda of the epididymis not observed NOS I immunoreactivity.
The specific localization of NOS I in buffalo epididymis suggest that nitric oxide may be involved to explain epididymal function: maturation and storage.

Key words*:* Buffalo*, Epididymis,* NADPH-d, NOS I.

INTRODUCTION - The buffalo epididymis was particularly studied in order to evidence the morpho-structural characteristics that affect the reproduction of this species, that even though is domesticated species still exhibits a seasonal sexual activity.
Paino et al. (1983) studied the water buffalo epididymis, and suggested that the vascular structure connecting the testis to the epididymis plays an important role in the transfer of the substances involved in sperm maturation. Moreover, Scala et al. (2002) investigated the microvasculature of the water buffalo epididymis and showed fenestrations that occupied ovoid inside the endothelium of the postcapillary venules. They connected the venules of the blood vascular system to the capillary of the peripheral lymphatic vascular system and appear to play an important role in the absorption and secretion processes of the epididymal epithelium.

In the present study, the presence of the neuronal nitric oxide (NOS I) in the buffalo epididymis have been evaluated by nicotinamide adenite dinucleotide phosphatase-diaphorase (NADPH-d) histochemichemistry to the light microscope (LM) and NOS immunoistochemistry to the scanning electron microscope (SEM), respectively.

METERIAL AND METHODS - The epididymis of 6 adult buffaloes were collected immediately after slaughter. The specimens were collected from the following regions: caput, corpus and cauda.

Histochemistry

The specimens were cut in small pieces. Samples were washed in 0.1 M PBS, transferred into a graded series of saccarose (10%, 20%, 30%), immersed in Tissue teck OCT compound, frozen in liquid nitrogen, and sectioned by a cryostat. In order to measure NADPHd activity, sections were incubated with 0.25 mg/ml nitro blue tetrazolium, 1 mg/ml NADPH, and 0.5% Triton X-100 in 0.1 M Tris-HCl buffer, pH 7.3, at 37°C for 10-15 min in a dark box or at room temperature for 30 min. The reaction was stopped by sample immersion in 0.1 M Tris-HCl buffer. Finally, sections were mounted on cover slips, examined under a light microscope, and photographed. Control sections included incubation in media in which substrate was omitted, and preincubation with the sulphydryl inhibitor, 5.5'-dithio-bis-2-nitrobenzoic acid. Neither of these controls produced positive immunosignals.

Immunogold-labeling SEM analysis

For the immunogold-labelling SEM analysis, the specimens were cut in small pieces, and immersed in PBS for 1 h. Samples were incubated for 2 h with a solution containing normal goat serum diluted 1:10 in PBS, and next with a primary polyclonal antibody directed toward nNOS, diluted 1:1500 in PBS, overnight at 4°C. After washing in PBS, the samples were incubated with gold-conjugated goat anti-rabbit IgG diluted 1:200 in PBS, for 1 h at room temperature. The secondary antibody was conjugated with gold particles of different size, namely 5 and 15 nm. After washings in PBS, samples were fixed by 2.5% glutaraldehyde in 0.1 M cacodilate buffer containing $CaCl_2$, pH 7.2, for 30 min. After the fixation step and washings with distilled water, samples were subjected to silver enhancement. The enhancement process enables the use of antibodies conjugated to smaller (1-5 nm) gold particles, preserving the advantage of faster penetration and higher labelling efficiency. Next, samples were dehydrated through an ethanol series, and dried to the critical point. The specimens, mounted on stubs, were examined under a LEO 435 VP scanning electron microscope at variable pressure (80-120 Pa) in the back-scattered electron mode which allows to detect gold particles associated to cells even if they are located intracellularly. The samples had not been coated by gold-palladium, so that the only conjugated gold deriving from immunocytochemical reaction was observed by SEM, and photographed.

RESULTS AND CONCLUSIONS - Histochemistry: examination of epididymis specimens revealed an intense NADPH-d staining in the basal cell epithelium and endothelium cells of blood vessel. The NADPH diaphorase staining was diffuse and granular only along the caput epididymal epithelium (Figure 1).

Figure 1. NADPH diaphorase of the buffalo epididymis. Strongly stained epithelium of epididymis (caput) is clearly visible.

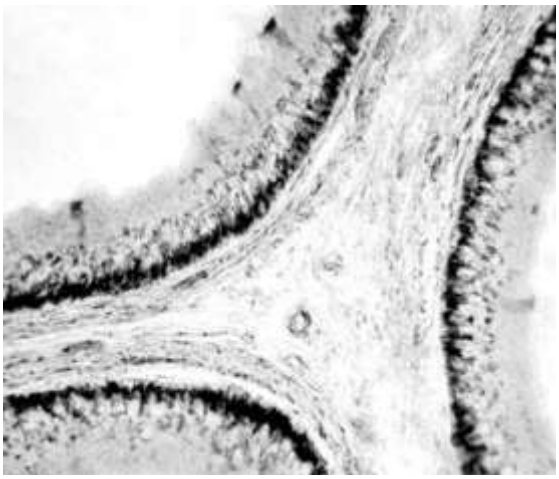
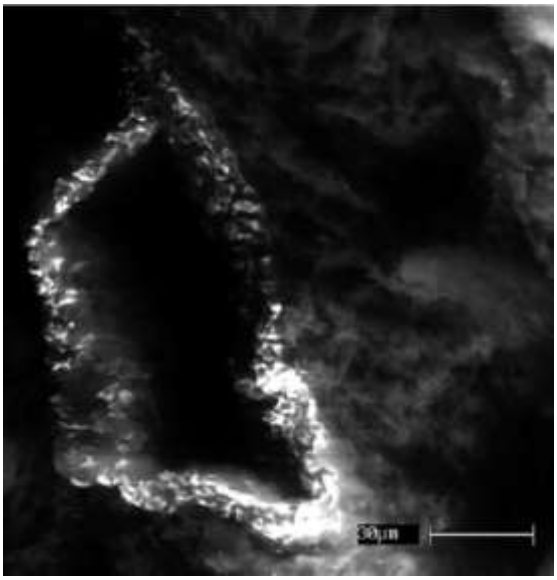


Figure 2. Scanning electron micrograph of nNOS immunorteactive cells of the buffalo epididymis. Immunoreactivity of epithelium (caput) is evident.



NADPH diaphorase staining was less intense or absent in the corpus and in the cauda of epididymis. Dense NADPH diaphorase is labeling in the endothelium of blood vessels along the whole buffalo epididymis.

Immunoistochemistry: intense NOS I immunoreactivity was detected in the caput epididymis specimen by immuno-SEM. The basal epithelium showed intense and wide-spread immunoreactivity. In the corpus and in the cauda of the epididymis not observed NOS I immunoreactivity (Figure 2).

The specific localization of NOS I in buffalo epididymis suggest that nitric oxide may be involved to explain epididymal function: maturation and storage. In addition nitric oxide (NO) is involved in spermatozoa capacitation. The importance of NO on sperm capacitation was also reflected on the level of tyrosine phosphorylation of sperm proteins. A correlation between sperm capacitation and tyrosine phosphorylation was observed: when capacitation was accelerated by a NO-releasing compound, there was an increase in tyrosine phosphorylation, whereas when sperm capacitating was inhibited by N^G-nitro-L-arginin methyl ester (L-NAME), there was attenuation in tyrosine phosphorylation of sperm proteins. Therefore, tyrosine phosphorylation of sperm proteins appears to be regulated by NO (Herrero *et al*., 1999). The biological significance of the tyrosine phosphorylation changes induced on exposure to calcium-depleted medium was demonstrated when these cells were subsequently shown to exhibit an enhanced capacity for undergoing the acrosome reaction, providing sufficient extracellular calcium was present to support the membrane fusion events associated with exocytosis (Baker et al., 2003).

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