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MAST CELLS IN AUTONOMIC GANGLIA AND NERVES AND IN SMALL BLOOD VESSELS IN THE RENAL HILUS OF DOMESTIC SWINE

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Summary

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After toluidine blue staining and polychromatic staining, mast cells were observed in autonomic ganglia and nerves on histological and semithin cross sections, as well as in the wall of small vessels located in the renal hilus, around the renal artery and vein and their first branches. The light microscopy revealed that mast cells were situated among the pseudounipolar nerve cells of ganglia, inside the autonomic fibres and in their perineurium. In small blood vessels, mast cells were located in the wall of arterioles and small arteries and near the wall of venules and small veins. Mast cells were also observed in the connective and adipose tissues of the renal hilus. The data of the present study allowed us to suggest that mast cells were involved in the function of mentioned structures via the biologically active substances, secreted by them.

Key words: ganglia, mast cells, nerves, renal hilus, small blood vessels, swine

INTRODUCTION

Mast cells are known to secrete biologically active substances – histamine, 5-hydroxytriptamine, dopamine, vasoactive intestinal polypeptide etc., some of them proven to be involved in nervous system activity. Therefore, the attention of investigators was focused on the possibility for functional communication between mast cells and the nervous system (Coupland & Heath, 1961; Falk *et al.*, 1964; Cutz *et al.*, 1978; Newson *et al.*, 1983; Wingren *et al.*, 1983; Dimitriadou *et al.*, 1987; Stead *et al.*, 1987; Barret & Pearce, 1991). Later, more data were gathered that offered a new insight into this functional communication after discovering that mast cells produce and release nitric oxide, superoxide and a nerve growth factor (Aloe *et al.*, 1994; Leon *et al.*, 1994; Sekar *et al.*, 2006). Despite the demonstrated close location of nerves to mastocytes and even a direct innervation of these cells (Newson *et al.*, 1983; Bienestock *et al.*, 1987; Dimitriadou *et al.*, 1987; 1994; Keith *et al.*, 1995; Mori *et al.*, 2002; Ito & Oonuma, 2006), there are only single reports about the presence of mast cells inside nervous structures (Gulubova *et al.*, 2000; Mori *et al.*, 2002). There are however no data about the localization of mast cells in nervous structures and the small blood vessels of the renal hilus in domestic swine. Our research during the last years evidenced convincingly the occurrence of mast cells in kidneys and renal vessels,

renal pelvis and ureter (Vodenicharov, 1995; Vodenicharov & Cirnuchanov, 1995a; 1995b; Vodenicharov & Chouchkov, 1999; Vodenicharov *et al.*, 2005a; 2005b; 2005c). We were able to observe mast cells in the renal hilus as well, some of them being in immediate vicinity to autonomic nerves around the large blood vessels.

These observations of ours as well as the lack of literature data about such a localization of mast cells inspired the present investigation aiming to elucidate more completely the role of mast cells in the kidney of domestic swine.

MATERIALS AND METHODS

The material for the study was obtained from 16, 8–10 month-old pigs from both genders (8 castrated males, 6 castrated females, 2 intact males), slaughtered for meat consumption in a slaughterhouse. Immediately after the slaughter and removal of kidneys, transverse sections were made through the renal artery and the renal vein with a part of surrounding tissues, and fixed in Carnoy's liquid for 4 hours. Material from both kidneys was also collected for transmission electron microscopy study. It was fixed by immersion in 2% glutaraldehyde in cacodilate buffer, washed in the same buffer and post fixed in 4% osmium tetroxide for 2 hours. The specimens were then washed in buffer, cleared in propylene oxide, in propylene oxide/Durcupan mixture and embedded in Durcupan (Durcupan, Fluka AG, Buchs SG, Switzerland). By means of an ultra microtome, semithin cross sections (1–2 μm) were prepared, that were polychromatically stained according to Humphrey & Pittman (1974). Some sections were stained using toluidine blue instead of methylene blue (unpublished modification of ours).

RESULTS

The light microscopy observations showed that mast cells were present in autonomic ganglia and autonomic nerves, around the renal artery and renal vein and their first branches. Furthermore, mast cells were observed near these structures and in immediate vicinity to small blood

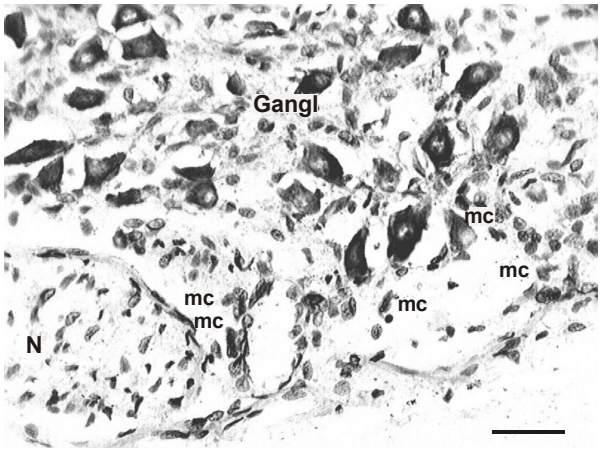

Fig. 1. Mast cells (mc) in an autonomic ganglion (Gangl). Some of them are situated among multipolar cells. N – autonomic nerve. Toluidine blue, bar = 50 μm.

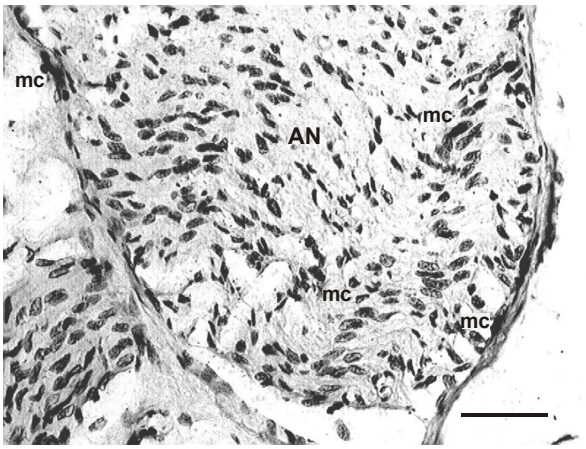

Fig. 2. Mast cells (mc) in the perineurium and inside an autonomic nerve (AN). Bar = 80 μm.

vessels of the renal hilus. Mast cells were also found out in the adventitia of renal artery and vein.

Mast cells inside the autonomic ganglia and nerves showed a well expressed γ-metachromasia after staining with 0.1% toluidine blue in McIlvane buffer (Fig. 1 and 2). On sections through autonomic ganglia, mast cells were observed freely among the multipolar nerve cells (Fig. 1). The analysis of mast cell occurrence in ganglia showed that in 9 studied sections with average length of 720±4.6 μm and width of 538±5.7 μm, 38.4±1.2 multipolar cells with nuclei and 8.6±0.5 mast cells were counted.

Single mast cells were observed in the perineurium of autonomic nerves (Fig. 2).

Mast cells found out in the connective and adipose tissues of the renal hilus were predominantly with an oval shape and in most of them, especially those stained by the modified technique of Humphrey & Pittman (1974) (methylene blue/toluidine blue, azur II, basic fuchsin), well visible cytoplasmic granules were observed. In most mast cells, the density of cytoplasmic granules was relatively high and that was the reason for the hardly visible cell nucleus (Fig. 3). The distribution of granules in the cytoplasm was regular.

The sections through nucleated mast cells, observed in immediate vicinity of the wall of small blood vessels (small arteries and veins, arterioles and venules) were mainly conic, less frequently fusiform. The cytoplasm of the major part of cells was also filled with well visible granules, best visualized on the semithin polychromatically stained sections (Fig. 4).

DISCUSSION

The present study demonstrates for the first time the occurrence of mast cells in autonomic ganglia and autonomic nerves of the renal hilus in the domestic pig. The localization of mastocytes both in the perineurium of nerve fibres and ganglia and inside them could be explained with transfer of these cells from the connective tissue around these structures. Also, no data are available for the presence of mast cells in the wall of small blood vessels in the

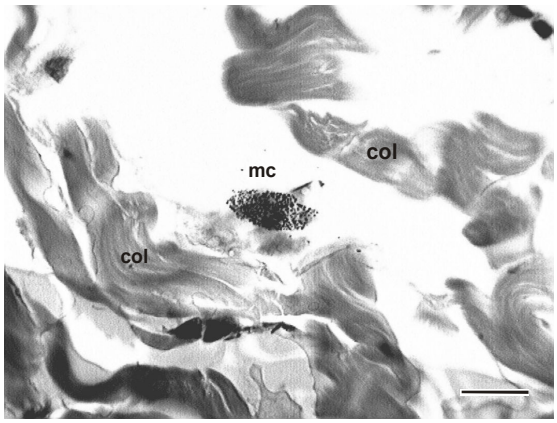

Fig. 3. Mast cells (mc) with well-defined granules in the connective tissue of the renal hilus among collagen bundles (col). Toluidine blue–azur II–basic fuchsin. Bar = 15 μm.

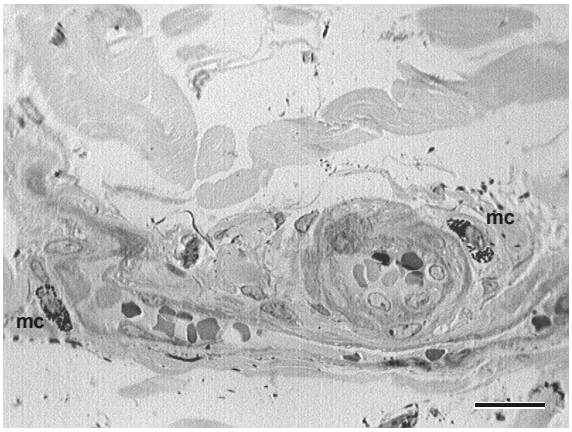

Fig. 4. Mast cells (mc) in immediate vicinity of an arteriole and a venule in the renal hilus. Methylene blue–azur II–basic fuchsin. Bar = 20 μm.

renal hilus, as well as in the adventitia of renal artery and vein in domestic swine.

The data about mast cells in kidneys of mammals and men are generally few. The considerable occurrence of mastocytes into the perivascular renal tissue in guinea pigs, reported by Ghanem *et al.* (1988), deserves however attention. It allowed assuming the existence of a potential functional relationship between mast cells and the autonomic nerve fibres in the perivascular tissue. A similar interpretation could be done to our findings about the presence of mast cells in the adventitia of large blood vessels, the renal pelvis and the ureter in swine (Vodenicharov, 1995; Vodenicharov & Cirnuchanov, 1995a; 1995b; Vodenicharov *et al.*, 2005b), because this tunica contains autonomic nerve fibres too. This view is further supported by data evidencing a close relationship between mast cells and peripheral nerve structures – mainly amyelinic and peptidergic nerve fibres, including primary sensory afferents

and autonomic ganglia (Keith *et al.*, 1995; Mori *et al.*, 2002; Ito & Oonuma, 2006).

The mast cells found in the wall of both small blood vessels and the adventitia of renal artery and vein contribute to the data about the occurrence of these cells in the renal vascular wall in domestic swine. The recently observed (Vodenicharov *et al.*, 2005b; Vodenicharov *et al.*, 2005c) histamine, vasoactive intestinal polypeptide and endothelin in mast cells, situated in the different layers of renal blood vessels and the ureter of domestic pigs, could explain the role of detected mast cells in the function of studied vessels.

It is well known that in the renal hilus, around the renal artery, the renal plexus, containing autonomic ganglia is located (Constantinescu *et al.*, 1992). The presence of mast cells in these ganglia and in the related autonomic nerves suggested that most probably, the substances released by mastocytes were neurotransmitters. On the other part, there remains the question about the reciprocity of the relations mast cells – nervous structures. Obviously, there is a need for more extensive enzyme- and immunohistochemical investigations for revealing the nature of biologically active substances in mast cells and the way of communication between them and the nervous structures surrounding the blood vessels of porcine renal hilus.

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