Bulgarian Journal of Veterinary Medicine (2012), **15**, No 4, 254–259

DISTRIBUTION AND DIMENSIONS OF NADPH-DIAPHORASE POSITIVE GANGLIONATED PLEXUSES IN PORCINE COMMON HEPATIC DUCT WALL

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Summary

Stefanov, I. S., 2012. Distribution and dimensions of NADPH-diaphorase positive ganglionated plexuses in porcine common hepatic duct. *Bulg. J. Vet. Med.*, **15**, No 4, 254–259.

The aim of the present study was to establish the expression of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity in ganglionated plexuses, their distribution and dimensions in mucosal, fibromuscular and subserosal layers of porcine common hepatic duct. The material was obtained from the common hepatic duct of 6 male and 6 female pigs. Tissue pieces were taken immediately after the slaughter and fixed in 4% paraformaldehyde. The NADPH-d histochemical expression was investigated according to the method of Sherer-Singler. The data showed that NADPH-d positive neurons and nitrergic autonomic nerves in the wall of the common hepatic duct obviously produced nitric oxide. Thus they were most probably involved in the regulation of the function of epithelium, blood vessels and the organ in general.

Key words: common hepatic duct, NADPH-d autonomic ganglia and nerves, swine

INTRODUCTION

Moncada *et al.* (1991) has characterized nitric oxide as a messenger molecule. The knowledge of the expression of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) of biliary tract is based mainly on data in guinea pigs (Siou *et al.*, 1993). In particular, nitric oxide (NO) possesses the same characteristic properties as the endothelium-derived relaxing factor (Furchgott & Zawadzki, 1980). It is suggested that NO plays a major role in the non-adrenergic and non-cholinergic neurotransmission of the gastrointestinal tract (Bult *et al.*,1990; Shuttleworth *et al.*, 1991), and also in guinea pig gallbladder (Siou *et al.*, 1993).

The anatomical and functional features of the biliary tract innervation are extensively studied in guinea pigs (Mawe & Gershon, 1989; Gonda *et al.*, 1995; Talmage *et al.*, 1996; Mawe, 2000; Mawe & Ellis, 2001) and the Australian brush-tailed possum (Padbury *et al.*, 1993a, 1993b; Meedeniya *et al.*, 2001, 2003).

Since the role of NO in the domestic pig common hepatic duct has not yet been examined, the aim of the present study was to determine the localisation and structural organisation of the NADPH-d-positive-ganglia and nerves in that organ.

MATERIALS AND METHODS

Animals

The material was obtained from the common hepatic duct wall of 6 male and 6 female pigs (Landrace×Bulgarian White), aged 6 months, slaughtered for meat consumption in a slaughterhouse in accordance with the Bulgarian laws (Anonymous, 2006).

Enzyme histochemical reaction for determination of NADPH diaphorase

The samples were immediately immersed in 4% paraformaldehyde (Sigma Aldrich Chemie, Switzerland) in phosphate-buffered saline (PBS), pH 6.9, for 8 h at 4 °C. Then the samples were removed and soaked in solution of 10% sucrose in PBS overnight. Sections of 15–20 μm thickness were prepared by means of a freezing microtome (Slee, Mainz, Germany). The free-floating sections were further processed according to the protocol of Sherer-Singler *et al.* (1983) by incubation in solution containing nitro blue tetrazolium (0.2 mg/mL, Sigma Aldrich Chemie GmbH, Germany), β-NADPH (Santa Cruz Biotech, Santa Cruz, CA, USA) and Triton X-100 (0.5%) (Merck Belgalabo, Overijse, Belgium) in PBS (0.1 M, pH 7.4) for 1–2 h at 37 °C.

Microscopic assessment of the reaction was scored as absent (0), weak (+), medium (++) and strong (+++).

Micromorphometrical investigation

The number of NADPH-d-positive ganglia per cross section and the number of NADPH-d positive neurons per ganglion were estimated in the wall of the common hepatic duct. The area, the width and the length of the ganglia, and the width and the length of the neurons in these ganglia were also measured. For that purpose a light microscope (ZEISS Primo Star, Germany), camera (Progres, Capture 2.6 - JENOPTIK) and software analysis programme (Soft Imaging System GmbH) were used.

Statistical analysis

Data for number and dimensions are given as mean ± SD. Statistical data processing was done using Student`s t-test (StatMost for Windows) and the difference was considered significant when P values were less than 0.05.

RESULTS

NADPH-d positive ganglia contained neurons in whose perikarya purple formazan deposits were observed. They showed a strong enzyme reactivity. Nuclei of neurons remained always unstained (Fig. 1). Ganglia were localised mainly in the subserosal layer. Their number was similar in male and female pigs (Table 1). The area of ganglia in males was a little bit smaller compared to females but without statistically significant difference (P>0.05).

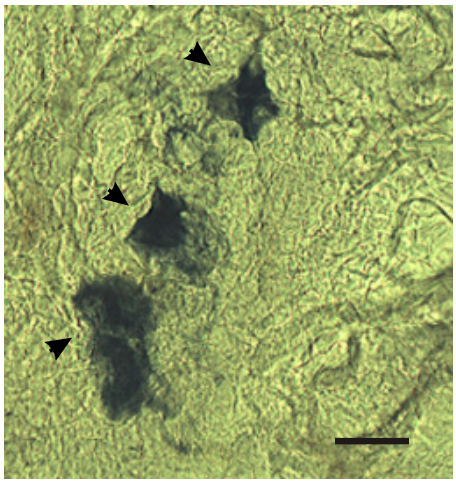

Fig. 1. NADPH-d positive ganglia (arrowheads) in the subserosal layer. Bar = 30 μm.

Table 1. Density (number of ganglia per cross section), area (μm^2), length (μm) and width (μm) of NADPH-d-positive ganglia in the wall of *ductus hepaticus communis* in 6-month-old pigs. Data are presented as mean ± SD (min-max range), n=6

Animals	Number of ganglia	Area of ganglia	Length of ganglia	Width of ganglia
Male pigs	5.02±0.75 (4.0–6.0)	12707.50±14800.97 3742.0 – 38123.0	188.02±66.72 (127.0–299.80)	93.20±84.89 39.7–238.7
Female pigs	5.20±0.73 (4.0–6.0)	13651.00±16435.80 (3699.0 – 41873.0)	188.50±67.85 (125.1–301.5)	92.15±83.80 (38.9–235.9)

Table 2. Density (number of neurons per ganglion), length (μm) and width (μm) of NADPH-d-positive neurons in the ganglia of *ductus hepaticus communis* in 6-month-old pigs. Data are presented as mean ± SD (min-max range), n=6.

Animals	Number of neurons	Length of neurons	Width of neurons
Male pigs	8.07±6.77 (1.0–21.0)	31.29±6.87 (15.9–41.2)	22.17±6.85 (12.2–32.0)
Female pigs	8.5±6.29 (1.0–20.0)	32.29±5.89 (16.4–41.9)	20.67±7.61 (12.1 – 33.10)

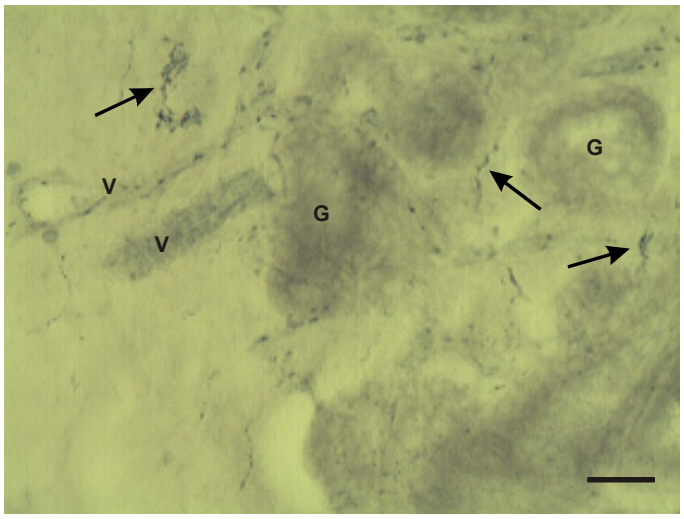

Fig. 2. Nitrergic nerves (arrows) localised near the NADPH-d positive blood vessels (V) and around glands (G) in the propria. Bar = 30 μm.

The density of neurons per ganglion and their dimensions were estimated as well (Table 2). Their number, length and width were similar in both genders.

Since statistical significant differences between males and females were detected neither in density, area, length and width of ganglia, nor in density, length and

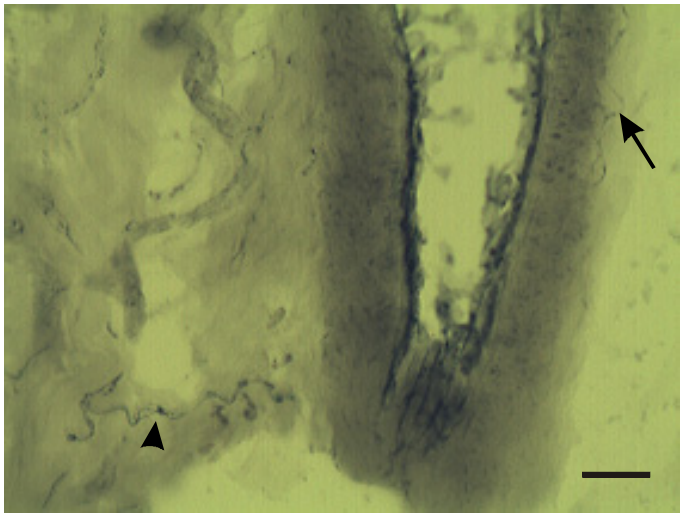

Fig. 3. Nitrergic autonomic nerves forming perivascular (arrow) and paravascular (arrowhead) nervous plexus. Bar = 30 μm.

width of neurons per ganglia in porcine common hepatic duct, sexual dimorphism was not established.

Nitrergic nerves showed strong to medium NADPH-d reactivity. They were with different dimensions and localised in the subepithelial, fibromuscular and subserosal layers of the common hepatic duct and around the glands (Fig. 2). The subepithelial plexus in the lamina propria was composed of a dense network of nerves close to the epithelium. Nitrergic nerves with medium to strong enzyme reactivity were observed near the blood vessels, forming peri- and paravascular nervous plexuses (Fig. 3).

DISCUSSION

In this study, the density, shape and dimensions of ganglionated nerve plexuses, as well as the dimensions and density of neurons per ganglion were estimated for the first time. Our findings indicated that the topographical and structural organisation of ganglia and nerves in the porcine common hepatic duct resembled the findings in the gall bladder of guinea pig, Australian brush-tailed possum and larger mammals (Balemba *et al.*, 2004). We found that the ganglionated nerve plexuses were mainly located in the subserosal layer of the porcine common hepatic duct, whereas in the gall bladder of Australian brush-tailed possum they were situated in the subepithelial, muscular and subserosal layers (Balemba *et al.*, 2004). Also, the ganglionated nerve plexuses in guinea pigs were situated in the subserosal and subepithelial layers but not in muscular layer. These results indicate that the structural organisation of the neural tissue in the porcine common hepatic duct was different from that of gall bladder in the guinea pig and the Australian brush-tailed possum. Our findings showed that in porcine common hepatic duct ganglia were situated only in the subserosal layer near the fibromuscular layer. These results support the findings in the gall bladder of guinea pig, where the subserosal

plexus was also composed of a network of small, irregular, triangular- or ovoid-shaped ganglia (Sutherland, 1967; Mawe & Gershon, 1989).

In the gall bladder of the Australian brush-tailed possum, nitric oxide synthase (NOS)-immunoreactive neurons in the ganglionated subepithelial plexus constituted the majority of the intrinsic secretomotor neurons (Meedeniya *et al.*, 2003). It is not yet clear whether gall bladder neurons and nerves can be functionally classified into muscle motor, vasomotor, secretomotor neurons, and interneurons as in the enteric nervous system. However, cholinergic gall bladder neurons can be separated into two distinct populations. Neurons expressing substance P, neuropeptide Y, and somatostatin are excitatory, whereas those expressing NOS, vasoactive intestinal polypeptide and pituitary adenylate cyclase activating polypeptide are inhibitory neurons (Talmage *et al.*, 1996; Mawe & Ellis, 2001). We suggest that the presence of NADPH-d positive ganglia is related to their role in the active relaxation of the common hepatic duct similarly to the gall bladder (Talmage & Mawe, 1993; Meedeniya *et al.*, 2001).

CONCLUSION

Our results showed that NADPH-d positive neurons and autonomic nerves in the wall of common hepatic duct obviously produced nitric oxide. Most probably they are involved in the regulation of the function of epithelium, blood vessels and organ functioning in general.

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Paper received 27.08.2012; accepted for publication 07.11.2012

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