http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 3(5), 759-762, 2000
© Copyright by the Capricorn Publications 2000

The Localization of NADPH-diaphorase Positive Cells and Autonomic Innervation of the Rat Thymus

F. Dorko[1], M. Kočišová[1], M. Sirotáková[2], K. Schmidtová[1], K. Lovásová[1] and E. Dorko[3]
[1]Department of Anatomy, Faculty of Medicine, P. J. Šafarik University, Košice, Slovak Republic, Europe,
[2]Institut of Experimental Medicine, Faculty of Medicine, P. J. Šafarik University, Košice, Slovak Republic, Europe,
[3]Physiology Institute, Faculty of Medicine, P. J. Šafarik University, Košice, Slovak Republic, Europe

Abstract: The localization of diaphorase activity of nicotinamide adenine dinucleotide phosphate (NADPH-d), acting as a marker of nitric oxide synthase (NOS), was studied in the thymus of rat. The NADPH-d active cells observed in the rat thymus were irregular with numerous projections and were located on the boundary of the cortex and the medulla. The thymus of the rat is innervated with parasympathetic and sympathetic nerve fibres. They are located as perivascular plexuses in the interlobular septa and on the corticomedullary border from where they extend to the organ parenchyma. The number and density of acetylcholinesterase (ACHE)-positive nerves increase up to the 3rd month of age and a reduction in thymic parenchyma and (ACHE)-positive nerves is visible in 12 month old rats. The density of adrenergic innervation increases up to the 3rd month and the adrenergic innervation of 12 month old rats corresponds to that observed in young rats.

Key words: adrenergic nerve structures, ACHE-positive nerve structures, autonomic innervation, NADPH-diaphorase activity, rat.

Introduction

The nicotinamide dinucleotide hydrogenphosphate diaphorase (NADPH-d) histochemical reaction consists in the transfer of hydrogen from the NADPH substate to tetrazolium salt which changes to insoluble deep - blue formazan Scherer-Singler *et al.*, (1983); Mizukawa (1990). The importance of this reaction increased after the discovery that the similarity of neuronal NADPH-d and nitric oxide synthase (NOS) is such that the two can be identical (Dawson *et al.*, 1991; Hope *et al.*, 1991). NOS is an enzyme responsible for nitric oxide (NO) synthesis by the conversion of L-arginine to citruline.

NO is a gas, therefore the free radical with a half - live of 5 seconds can easiby diffuse into tissue (Kiechle and Moliski, 1993). Its detection is possible by determining the NOS enzyme. Numerous studies proved that both forms of NOS – endothelial and neuronal – are located, together with NADPH-d, in the tissues with 4% paraformaldehyde.

The increasing amount of data indicates that NO mediates certain functions in the autonomous nervous system Grozdanovic *et al.*, (1992).

Our histochemical investigation of NADPH-d cells is concentrated on the presumed presence of NO in the thymus of rats.

Thymus, the primary lymphopoietic organ, affects decisively the normal development of the cell-mediated immune system (Sell, 1980). This was prooved by Good *et al.* (1962) after thymectomy on mice and rabbits. After puberty, involution of the thymus occurs with increasing age in mammals including the man (Singh, 1984). The normal function of the immune system and of T cells in particular declines with age (Weksler *et al.*, 1978). The autonomous innervation of lymphopoietic organs mediates an important influence on the immune function and because of that considerable attention is paid to neurovascular relations in these organs

The number and density of ACHE-positive nerves increase up to the 3rd month of age and a reduction in thymic parenchyma and ACHE-positive nerves is visible in 12- month-old rats (Sirotáková and Škardová, 1999).

The highest density of adrenergic and ACHE-positive nerves was observed in the period between 3rd and 4th month of age. After this period gradual reduction of adrenergic and ACHE-positive nerve structure was observed and the bursa cloacalis of a 12 months old chicken and pheasant showed only single nerve structures located mainly in the perivascular topography. (Kocišová *et al.*, 2000). The bursa of Fabricius and the thymus, the central lymphoid organs of birds, are esential for the development of peripheral lymphoid tissue and adaptive immune function. Both bursal-B and thymic-T –avian lymphocytes are very sensitive to apoptosis induced by immunosuppressive status (Škardová *et al.*, 1999).

Histological studies conducted in many workplaces have prooved the presence of autonomous nerves in both the perivascular topography and parenchyma of lymphopoietic organs (Bulloch and Pomerantz, 1984; Felten *et al.*, 1987; Zibers and Novotny, 1992).

Our interest in the influence of autonomous nerve system on the functional capacity of lymphopoietic organs induced us to study the adrenergic and cholinergic innervation of the rat's thymus in the postnatal period.

Materials and methods

The localization of NADPH-d positive cells in the thymus was studied 10 male (Wistar) rats. A histochemical reaction modified according to Scherer-Singler (1983) was used to visualize NADPH-d activity. The sections mounted in Entellan and evaluated under a light microscope.

Thymuses of 30 male and female (Wistar) rats, divided into three groups (1st group–rats from one day to three months of age, 2nd group – rats three to five months old, 3rd group–rats from five months to one year of age) were examined. The rats were killed by aether narcosis. Their thymuses were removed and tissue sections were cut in the cryo–cut (without fixation). The individual sections were then incubated in glyoxylic acid solution by means of histofluorescence method a modification of Shvalev and Zhuchkova (1987). In the study of ACHE-positive nerve profiles the tissue sections were placed in 4 % paraformaldehyde for 2–10 h. ACHE-positive nerve were observed by means of the direct tiocholine method modified by El Badawi and Schenk (1967). The individual sections were incubated for varying times (2–6 h).

The microscopic examinations and photo documentation were performed using the Jenalumar 2 (Zeiss, Jena).

Results

The NADPH-d histochemistry of the thymus of rats showed different distribution of diaphorase positive reactivity in the medulla and the cortex which appeared as deep blue – stained cellular structures. Diaphorase positive cells exhibited only slight intensity of colouration and diffuse distribution throughout the entire parenchyma cortex.

The NADPH-d positive cells were mostly seen an the medullary side of the corticomedullary function with few scattered in the inner aspect of the medulla (Fig. 1). Unlike the other rodents thymus, however, the NADPH-d positive cells in the rat appeared to be arranged in a band –like fashion at the periphery of the medulla. Intensivelly coloured cells were observed predominantly in the corticomedullary area where they occurred as irregular aglomerates, they exhibited a high degree of irregularity with numerous projections without NADPH-d positive nerve fibres.

ACHE-positive nerve fibres were observed most frequently in close relationship with interlobular perivascular plexuses. They consisted of thick ACHE-positive nerve fibres or fine nerve fibres. Solitary nerve fibres detached from the septal periarterial plexuses and entered the cortex of the thymus lobuli as intralobular ACHE-positive nerve fibres. Periarterial nerve plexuses were observed also in the corticomedullary region. The nerve fibres extending from them entered the cortex and medulla of the thymic lobuli. The number of ACHE-positive nerve fibres increased in the postnatal period up to the age of 3 months (Fig. 2). After this time a gradual reduction in the thymic parenchyma was observed, particularly in the cortex, which resulted in the reduction in ACHE-positive nerve structures. Examination of a 12-month-old rat showed considerably reduced ACHE-positive nerve fibres and thymic parenchyma.

The adrenergic nerve profiles were most frequently observed as periarterial nerve plexuses running between interlobular septa up to their terminal sections located at the level of the medulla or on the boundary of the medullary and cortical layers and rarely in the cortex. Numerous nerve plexuses were seen on the boundary between the medullary and cortical layers. The majority of them passed to the medulla and only a negligible portion entered the cortical layer.

However, the distribution of adrenergic nerve fibres in individual parts of the lobuli was not uniform. Some regions were relatively abundant with adrenergic nerve fibres while only few specifically fluorescent fibres or none at all could be observed in others.

The rats of the first group (up to 3 months of age) showed visible periarterial nerve plexuses in interlobular septa. The individual fibres were thin and contained distinct fluorescent varicose dilatations. These fibres formed a conspicuous portion of the microscopical picture. On the contrary, the solitary nerve fibres were present in limited numbers, mostly on the corticomedullary border. They exhibited the specific fluorescence of a non-uniform intensity (Fig. 3).

Along with the growth and development of the organ, from 3 weeks up to 5 months of age, the number and density of nerve components increased too. The fibres became thicker and more undulating and exhibited a more intensive specific fluorescence. The present periarterial nerve plexuses were abundant in intensively fluorescent fibres. The number of solitary nerve fibres increased too and their specific fluorescence became more intensive on both the corticomedullary border and in the medulla (Fig. 4).

The microscopical findings in the thymuses of older rats examined (5 months to 1 year old) were essentially the same, although they contained increasing amounts of adipose tissue which frequently hampered the identification of thymus structures. Nerve profiles became larger and the fluorescence was mainly by characterized thicker plexuses. However, in the dilated thymic septa of a

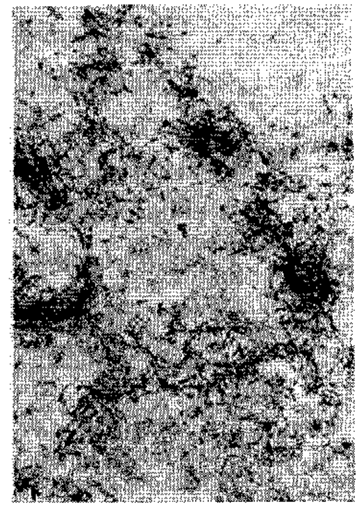

Fig. 1: NADPH-diaphorase positivity at the cortocomedulla junction of the rat thymus. Magn. x 120

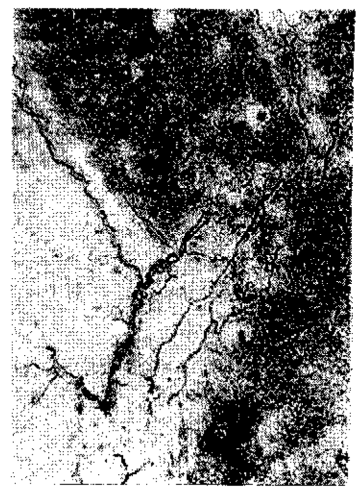

Fig. 2: ACHE-positive nerve plexuses and fibres in th corticomedullary region of the thymus lobulus. Thymu of a 3 – month old rat. Magn. x 120

Fig. 3: Adrenergic nerves in the perivascular topography whic are founded in the septum of the thymus of a 1 mont old rat. Magn. x 120

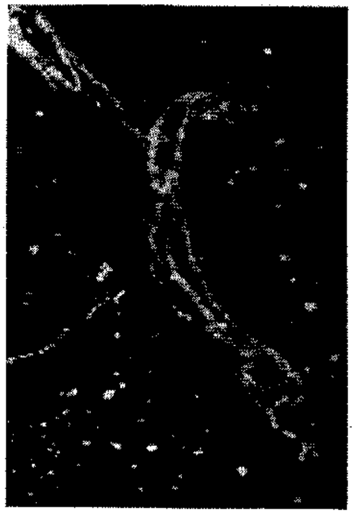

Fig. 4: Perivascular adrenergic nerve profiles located on the boundary of the cortex and medulla. Fine nerve fibres passing to the medulla detached from thymus of 5 month old rat. Magn. x 120

1-year-old rat the specifically fluorescent adrenergic nerve fibres were found routinely also along the lengthwise-running arterial branches.

Discussion

Our study engaged in the histochemical investigation of NADPH-d activity in the thymus of rats. Papers published on this subject stated that NADPH-d can be considered as a marker of NOS, the enzyme responsible for NO synthesis Dawson *et al.* (1991). The proof of activity of this enzyme in the nervous system of mammals was presented by Aimi *et al.* (1991); Valtschanoff (1992); Gulati *et al.* (1993); Downing (1994) described distribution of NADPH-d positive structures in the thymus of rats and chickens; Dorko *et al.* (1998) in the thymuses of pheasant and mice.

Our observations on the thymus of rats agree with the results mentioned above. The NADPH-d positive cells were observed as irregular agglomerates the bondary of the cortex and the medulla. Individual cells had irregular shapes and numerous projections. Their presence and localization allow us to presume that NO can participale in the neurotransmission in the thymus.

The rats thymus as in all mammals is innervated with both sympathetic and parasympathetic nerve fibres.

The first signs of ACHE-positive nerves in the thymus of 18-day-old rat foetus were described by Bulloch and Pomerantz (1984). Histochemical and ultrastructural studies proved that the internal distribution of ACHE-positive nerve fibres from the *nervus phrenicus* and *nervus laryngeus recurrens* were limited to subcapsular regions while the ACHE-positive fibres from the *nervus vagus* ended as abundant plexuses in corticomedullary thymic lobuli regions (Bulloch and Pomerantz, 1984).

The presence of ACHE-positive nerve structures in the rat's thymus from day 1 of age up to 18 months of the postnatal period was described by Kocišová *et al.* (1992), Sirotáková and Škardova (1999). They observed their highest density in the period between 8 and 10 weeks of age.

Our observations proved the presence of ACHE-positive nerves in the rat's thymus in the form of perivascular plexuses found in interlobular septa and on the corticomedullary border. Their number gradually increased from day 1 up to 3 months of age, i.e. up to sexual maturity. The 12-month-old rat examined in our study exhibited visible reduction in both the parenchyma and ACHE-positive nerve fibres. Our results confirm the findings of other authors suggesting the presence of ACHE-positive nerves in the thymic parenchyma.

Our findings about the adrenergic innervation in rats agree with those published in the literature (Felten *et al.*, 1987; Singh, 1984). The noradrenergic postganglial sympathetic nerve fibres supply the vascular system, smooth musculature, and parenchyma of the organ, namely the T-dependent plasmocyte-abundant parts.

The presence of noradrenalin receptors on vessel walls is well known. According to Cowen (1984) the closeness of noradrenalin-containing nerves and smooth muscles is associated with functional neurotransmission and the increased effect of noradrenalin on smooth muscles of vessels can also affect the circulation of lymphocytes.

The papers published on this subject mentioned the reduction of sympathetic nerves in the lymph nodes and spleen of old mice and rats. On the other hand, no decline was observed in the thymus (Felten *et al.*, 1987). An increase in the number of sympathetic nerve fibres during ontogenesis was observed by Singh (1984). Their maximum was reached at sexual maturity. The author stated that this increase in the number of sympathetic nerves could induce involution of the thymus. The adrenergic innervation of the rat's thymus increased up to the age of 3 months in a similar way as the ACHE-positive innervation observed by Bulloch and Pomerantz (1984) and Dorko *et al.* (1994). These nerve fibres were thicker in the older rats. However, an increase in the density of nerve fibres with progressing involution of the organ (Zibers and Novotny, 1992), observed in our experiment, was less noticeable.

In conclusion, in agreement with other authors, one can state that both the adrenergic and ACHE-positive nerve structures participate in the innervation of the thymus. An antagonistic influence of the two basic components of the efferent autonomous nerve system, *sympathicus* and *parasympathicus,* was observed in experiments with mice (Singh and Fatani, 1988).

References

Aimi, Z., M. Fujimura, S.R. Vincent and H. Kimura, 1991. Localization of NADPH-diaphorase containing neurons in sensory ganglia of the rat. J. Comp. Neurol., 306: 382-392.

Bulloch, K. and W. Pomerantz, 1984. Autonomic nervous system innervation of thymic - related lymphoid tissue in wildtype and nude mice. J. Comps. Neural., 228: 57-68.

Cowen, T., 1984. An ultrastructural comparison of neuromuscular relationships in blood vessels with functional and non-functional neuromuscular transmission. J. Neurocyt., 13, 369-392.

Dawson, T. M., D.S. Bredt, M. Fotuhi, P.M. Hwang, S. H. Synder, 1991. Nitric oxide synthase and neuronal NADPH-diaphorase are identical in brain and peripheral tissues. Proc. Natl. Acad. Sci. USA, 88: 7797-7801.

Dorko, F., A. Gomboš and M. Kocišová, 1994. Developmental of ACHE-positive innervation of the rat thymus during prenatal and postnatal period. Funct. Develop. Morphol., 4: 139-140.

Dorko, F., M. Kocišová, A. Gregor and E. Dorko, 1998. Localization of NADPH-d positive cells in thymuses of pheasant and mice. Brat. Lek. Listy, 99: 104-108.

Downing, J. E. G., 1994. Multiple nitric oxide synthase systems in adult rat thymus revealed using NADPH-diaphorase histochemistry. Immunology, 82: 659-664.

El Badawi, A. and E.A. Schenk, 1967: Histochemical methods for separate, consecutive and simultaneous demonstration of acetylcholinesterase and norepinephrine in cryostat sections. J. Histochem. Cytochem., 15: 580-588.

Felten, S. Y., D.L. Bellinger, T.J. Collier, P.D. Coleman and D.L. Felten, 1987: Decreased sympathetic innervation of spleen in aged fischer 344 rats. Neurobiol. Ageing, 8: 159-165.

Good, R. A., A.P. Dalmasso, C. Martinez, O.K. Archer, J.C. Pierce and B.W. Papersmaster, 1962: The role of the thymus in the development of immunologic capacity in rabbits and mice. J. Exp. Med., 116: 773-798.

Grozdanovic, Z., H.G. Baumgarten and G. Bruning, 1992: Histochemistry of NADPH-d, a marker for neuronal nitric oxide synthase in the peripheral autonomic nervous system of the mouse. Neuroscience, 48: 225-235.

Gulati, P., A.S. Chan and S.K. Leong, 1993: NADPH-diaphorase positive cells in the chick and rat thymus. Thymus, 22: 117-124.

Hope, B. T., G.J. Michael, K.M. Knigge and S.R. Vincent, 1991. Neuronal NADPH-diaphorase is a nitric oxide synthase. Proc. Natl. Acad. Sci., USA, 88: 2811-2814.

Kiechle, F. L. and T. Maliski, 1993. Nitric oxide biochemistry, pathophysiology, and detection. Am. J. Clin. Pathol., 100: 567-573.

Kocišová, M., A. Gomboš and F. Dorko, 1992. Innervation of the rat thymus. Folia Fac. Med. Univ. Šafarik., Cass. , XLIX, 53-58.

Kocišová, M., M.F. Sirotáková, F. Dorko, K. Schmidtová, S. Rybárová and I. Škardová, 2000. The autonomic Innervation of the Bursa Cloacalis of Chickens and Pheasants. Pakistan J. Biol. Sci., 3: 236-238.

Mizukawa, K., 1990. Reduced nicotinamide adenine dinucleotide phosphate - diaphorase histochemistry: Light and electron microscopic investigations. In Methods in Neuroscience, 3, Ed. Academic Press. Inc., 457-472.

Sell, S., 1980. Immunology, Immunopathology and Immunity. Harper and Row, Hagerstown, Maryland, pp: 77-78.

Shvalev, V. N. and N.J. Zhuchkova, 1987. An improvement in histochemical findings in adrenergic nervous elements in glyoxylic acid solutions with the aid of dimethylsulphoxide. Ark. Anat., 93: 91-92.

Scherer-Singler, U., S.R. Vincent, H. Kimura, E.G. McGeer, 1983. Demonstration of a unique population of neurons with NADPH-diaphorase histochemistry. J. Neurosci. Methods, 8: 229-234.

Singh, U., 1984. Sympathetic innervation of the foetal mouse thymus. Eur. J. Immunol., 14: 757-759.

Singh, U. and J. Fatani, 1988. Thymic lymphopoiesis and cholinergic innervation. Thymus, 11: 3-13.

Sirotáková, M. and I. Škardová, 1999. The innervation of the thymus of the rat. Folia Veterinaria, 43: 144-146.

Škardová, I., F. Ojeda and M. Levkut, 1999. Various kinds of immunosuppressive factors, that induce apoptosis in bursal B and T lymphocytes in chickens. The Journal of Scanning Microscopy, 21: 104-105.

Valtschanoff, J. G., R.J. Weinberg and A. Rustioni, 1992. NADPH-diaphorase in the spinal cord of rats. J. Comp. Neurol., 321: 209-222.

Weksler, M. E., J.B. Innes and G. Goldstein, 1978: Immunological studies of ageing IV. The contribution of thymic involution to the immune deficiencies of ageing mice and reversal with thymopoietin. J. Exp. Med., 148: 996-1006.

Zibers, T. and G.E.K. Novotny, 1992: Quantification of thymic innervation in juvenile and aged rats. Acta Anat., 145, 283-288.