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Localisation of NADPH-diaphorase-positive structures in the thymus of the rat, mouse and rabbit

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Localisation of the diaphorase activity of nicotinamide adenine dinucleotide phosphate (NADPH-d), acting as a marker of nitric oxide synthesis (NOS), was studied in the thymus of the rat, mouse and rabbit. The NADPH-d-active cells observed in the rat thymus were irregular in shape with numerous projections and were located on the boundary between the cortex and the medulla. The NADPH-d--active cells in the thymus of the mouse were located predominantly in the medulla. They varied in coloration and their shape was oval, round, or irregular. NADPH-d-positive nerve fibres were located perivascularly. The rabbit thymus displayed a lightly stained cortex, whereas the medulla was seen as a rounded complex of intensively stained cells, without sharp demarcation between them. In the rat thymus, the NADPH-d-positive nerve fibres were not evident, whereas NADPH-d-positive nerve fibres were seen in the perivascular topography of the mouse and the rabbit thymus.

These results suggest that NO may participate in the regulation of the thymic function in the species. In summary, the present results reveal the distribution of NADPH-diaphorase-d-positive structures in the rat, mouse and rabbit thymus.

key words: NADPH-d, thymus, rat, mouse, rabbit

INTRODUCTION

The nicotinamide dinucleotide hydrogenphosphate diaphorase (NADPH-d) histochemical reaction consists in the transfer of hydrogen from the NADPH--substrate to tetrazolium salt which changes to insoluble deep-blue formazan [19, 21]. 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 were identical [8, 16]. Nitric oxide (NO) is synthesised from L-arginine by an enzyme nitric oxide synthase (NOS), which can exist in a number of isoforms one of them being the neuronal NOS. It has been found that the neuronal NOS, present in the brain and peripheral organs, is identical to NADPH-d [16]. With regard to the increasing importance of NO in the organism, the histochemical method for NADPH-d also appears suitable for the study of NOS-possessing nerve structures. Numerous studies have demonstrated that both forms of NOS, the endothelial and the neuronal, are located, together with NADPH-d, in tissues fixed with 4% paraformaldehyde. An increasing amount of data indicates that NO mediates certain functions in the autonomous nervous system [4, 14].

The aim of the work was to study and compare the NADPH-d activity of cells in the thymuses of var-

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ious species of rodents and to identify their shape differences and localisation in individual components of the parenchyma.

MATERIAL AND METHODS

Experiments were carried out on 10 mice of both sexes, of 40–50 g body weight, 8 male (Wistar) rats weighing 250–300 g and 9 Chinchilla rabbits, body weight 2.5–3.0 kg of both sexes. Before experiments, the animals were reared in the Central Animal Husbandry quarters under veterinary care. The animals were anaesthetised by pentobarbital and killed by intracardial perfusion of saline solution and subsequent perfusion of 4% paraformaldehyde containing 0.1% glutaraldehyde in 0.1 M phosphate buffer of pH 7.4. The solutions were prepared immediately before perfusion. After the perfusion, the thymuses were removed surgically and stored in identical fixative solution for 3–4 hours. They were then transferred to 30% saccharose solution in the same phosphate buffer and stored overnight at 4°C. Sections of 43–45 ~m were prepared by means of a cryomicrotome.

A histochemical reaction modified according to Scherer-Singler et al. [21] was used to visualise NADPH-d activity. The sections were incubated in a solution of 1.5 mM tetrazolium nitroblue (NBT, Sigma Chemicals, N-6876), 1.0 mM beta nicotinamide adenine dinucleotide phosphate (NADPH, Sigma Chemicals, N-1630), 10.0 monosodium maleate (maleic acid, Sigma Chemicals, N-1125) and 0.5 Triton X-100, dissolved in 0.1 M phosphate buffer, pH 8.0, at 37°C for hour. After the incubation, the sections were washed with 0.1 M phosphate buffer (pH 7.4), mounted in Entellan and evaluated under a light microscope.

RESULTS

The NADPH-d histochemistry of the thymus of rodents showed a different distribution of diaphorase-positive reactivity in the medulla and the cortex, which appeared as deep blue stained cellular structures.

Thymus of rats

The diaphorase-positive cells exhibited only slight intensity of coloration and diffuse distribution throughout the entire parenchyma of the cortex. The distribution of NADPH-d positive cells in the thymus medulla of rats is different. Intensively coloured cells were observed predominantly in the corticomedullary area where they occured as irregular agglomerates (Fig. 1). They were only sporadically dispersed in the inner cortex. In comparison with the cells of the thymus medulla in mice they exhibited a high degree of irregularity with numerous projections without NADPH-d-positive nerve fibres.

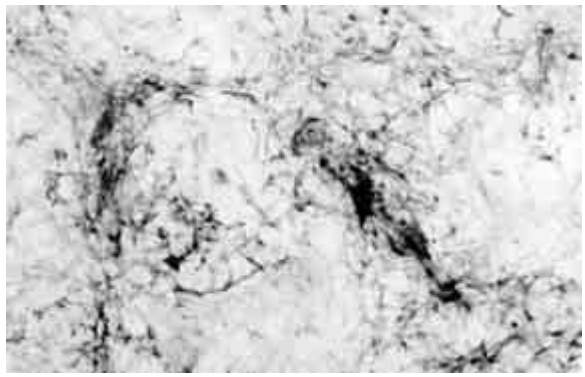

Figure 1. NADPH-d-positive cells on the border of the cortex and medulla in the rat thymus. They are of different shape and of intensive coloration. Primary magnification: ×120.

Thymus of mice

The NADPH-d-positive cortex cells occurred in low numbers and diffuse dispersion. Individual cells were oval and exhibited a low intensity of diaphorase-positive coloration.

No projections were observed in these cells. Examination of preparations are of better visibility only for radialy oriented vessels.

The thymus medulla of mice contained more NADPH-d positive cells (Fig. 2). The shape of these cells was similar to that observed in the cortex. They were mostly oval, round and also irregular in shape. They varied in the intensity of their coloration. Medullary cells showed a marked intensity of NADPH-d activity. They were of irregular shape and dark colour.

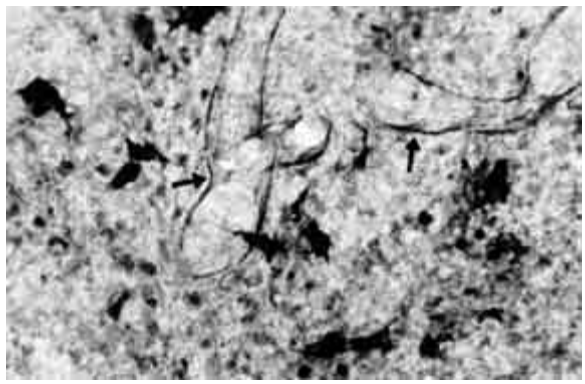

Figure 2. NADPH-d-positive cells in the medulla of the mouse thymus. Fine NADPH-d-positive nerve fibres are observed in the perivascular area. Primary magnification: × 120.

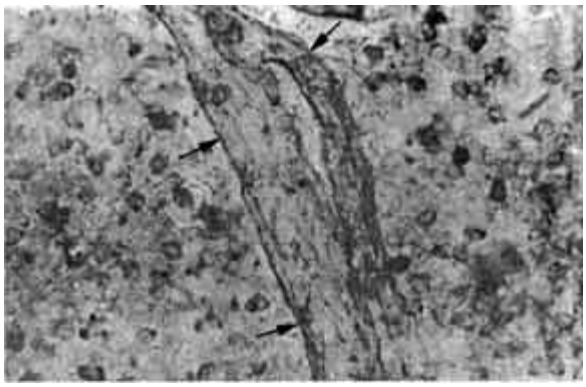

Figure 3. Detailed view of NADPH-d-positive cells of the rabbit. NADPH-d-positive nerve fibres arranged in perivascular topography. Primary magnification: × 120.

The cells had short projections and were frequently located close to the vessels. Blood vessels located in the medulla and septa showed signs of NADPH-d-positivity of the endothelial cells. NADPH-d-positive nerve fibres were observed in perivascular localisation.

Thymus of rabbits

The NADPH-d-positive cells were present in the cortex and the medulla of the thymus, but there were more of these cells in the medulla. The cells of the cortex were rounded or irregular in shape and were only slightly labelled, solitary and scattered. The medulla was characterised by the presence of a large quantity of cells with varying density of staining (Fig. 3). In general, they were similar in form and the oval, rounded, monopolar type of cell bodies in particular were observed to lack any apparent processes. Blood vessels localised in the septa showed signs of NADPH-d-positivity of the endothelial cells. NADPH-d-positive fibres were observed in perivascular localisation.

DISCUSSION

In our study we described the NADPH-d-positive structures in rodent thymuses. The NADPH-d activity could be regarded as a marker of NOS, the enzyme responsible for the synthesis of NO [5, 8, 22]. The proof of activity of this enzyme in the nervous system of mammals was presented by the distribution of NADPH-d-positive structures described in the thymus of rats and chickens [1, 3, 13–15, 26]. Dorko et al. [11] studied the localisation of NADPH-d positive cells in the thymuses of mice and pheasants.

Our observation of the thymus of rats agrees with the results mentioned above. The NADPH-d-positive rat cells were observed as irregular agglomerates on the boundary of the cortex and the medulla. Individual cells had irregular shapes and numerous projections. In our observations of NADPH-d-positive cells in the thymus of mice we have demonstrated differences in the number and type of these cells in the cortex and the medulla. While oval, diffuse, dispersed cells of a low intensity of coloration predominated in the cortex, the medulla contains a higher number of various types of NADPH-d-positive cells. They occurred in numerous agglomerations with varying intensity of coloration. Attention should be paid to cells that are dark in colour, of irregular shape and which contain small projections. These were frequently located in close proximity to vessels such as NADPH-d-positive nerve fibres. The cortex of the rabbit thymus showed only weak NADPH-d staining, similar to the thymic cortex of other animal species [14]. In contrast, the intensity of NADPH-d-positive labelling was greater in the medulla. Various types of cells were observed, recognisable according to the density of NADPH-d staining. This unequal staining may suggest an unequal utilisation of NO by these cells. The data presented by [2] indicates that lymphocyte functions appear to be dependent on L-arginine, the NO pathway. NADPH-d-positive cells of the medulla formed rounded oval groups, easily distinguishable at low magnification. One type of these cells was morphologically similar to those weakly stained in the cortex.

In this study our interest was focused mostly on the presence of some NADPH-d-positive nerve structures. However, these nerve fibres ran alongside blood vessels. The innervation of the thymus has previously been clearly demonstrated [6]. The non-adrenergic and non-cholinergic (MANC) neurotransmission in the peripheral nervous system has been discussed by other authors [7, 27]. The results of [20] suggest that NO may participate in the haemodynamic control of the pancreas, due to NADPH-d activity observed in both its endocrine and exocrine aspects. Similarly, in the thyroid gland NADPH-d-labelled neuronal bodies, nerve fibres and vascular endothelium were discovered [25].

By analogy with other peripheral targets, the possibility exists of the presence of NANC fibres among thymic innervation. Our results are able to support this possibility in the case of the mice and rabbit thymus, although no NADPH-d-positive nerve fibres have been demonstrated in the rat and chick thymus [15]. Our observation of the NADPH-d activity of nerve fibres in the mice and rabbit thymus disagrees with those of others, who have investigated NADPH-d activity in the above-mentioned animal

species. There is no clear explanation for this discrepancy, except for interspecies differences. In support of this view [20] it can be stated that interspecies differences were evident in the distribution of NADPH-d-positive nerve fibres in the pancreas, suggesting that NO may exert different modes of action on the pancreas from species to species. It should be noted, that for final proof of thymic NANC fibres, it will be necessary to carry out more experiments using other methods.

In summary, the present results reveal the distribution of NADPH-diaphorase-positive structures in the rat, mice and rabbit thymus. Especially remarkable were the blood vessels with clearly visible endothelial cells and with fine perivascular nerve fibres. These findings suggest, that NO may participate in the regulation of the thymic function.

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