NADPH-Diaphorase-Containing Neurons in the Medullary Structures Involved in Generation of the Respiratory Activity in Neonatal Rats

D. V. Volgin, V. A. Maiskii, D. A. Vasilenko, and M. M. Seredenko

INTRODUCTION

Currently one of the techniques for visualization of neurons containing the enzyme nitric oxide synthase (NO synthase, NOS) is histochemical identification of another enzyme, diaphorase of the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH-d [1-4], which either is identical to NOS or can be regarded as an acceptable marker for it [1-3]. This is confirmed by the data about NADPH-d-immunoreactivity of the neurons from various brain structures containing immunohistochemically identifiable NOS [4]. In particular, it was shown [5] that distributions of NOS-immunoreactive and NADPH-d-positive neurons in the medulla of rats are practically identical. The level of NOS activity and the number of NOS-immunoreactive neurons in that brain region are not very high [5, 6]; however, NO should be considered an important component of the medullary mechanisms of the respiratory and cardiovascular systems [7, 8]. Our earlier data showed that endogenous NO is actively involved in regulation of the respiratory activity generated by semi-isolated medullo-spinal preparations (SIMSP) of early postnatal rats, under both normal and hypoxic conditions [9, 10].

Localization of NADPH-d-positive and NOS-immunoreactive neurons was studied in adult animals [2, 4]. Such cells were identified within the medullary structures of the respiratory generator: in the nucl. tractus solitarii, nucl. ambiguous, and nucl. paragigantocellularis lateralis [11]. The brainstem of newborn animals has not been studied in this respect. It was reported that within the earliest postnatal stages the activity of NOS is near zero, and it becomes noticeable only on the 10th
postnatal day. This is why we tried to identify NADPH-d-positive neurons in the medullary generator structures of early postnatal rats.

METHODS

Experiments were carried out on 8 4-day-old Wistar rats. NADPH-d-reactive structures were identified using a modified technique of Vincent and Kimura [2, 3] in frontal slices of the medulla and 3 cervical spinal segments.

Under deep ether anesthesia, we opened the posterior cranial dimple under continuous irrigation of the operation field with the modified cooled (10°C) Krebs solution (the solution composition was described earlier [9, 10]). The cerebellum was aspirated, and the medulla was superfused with a fixative (4-5% paraformaldehyde solution in 0.1 M phosphate buffer, PB; pH 7.3). After a 10- to 15-min-long superfusion, the brainstem was taken off, postfixed for 2 h in the same fixative at 4-5°C, and exposed for 24 h to a 15% sucrose solution. Using a cryostat, 50-μm-thick frontal slices were prepared, rinsed for 1 h in PB, and histochemically processed for during 40-60 min at 37°C. The medium contained: 0.1 M PB, 0.3% triton X-100, 0.5 mg/ml tetrazolium blue, 1.0 mg/ml NADPH sodium salt, and 1.2 mg/ml sodium malate (pH 7.3). All substances were produced by Sigma, USA. The slices were rinsed in PB, mounted on gelatin-covered glasses, and dried at room temperature. Then they were dehydrated and clarified being passed through absolute alcohol and xylol, embedded in Canada balsam, and photographed with the use of a light microscope. Localization of NADPH-positive cells was indicated on the schemes of frontal sections using a two-coordinate plotter. Borders of the nuclei and tracts were identified according to the coordinates of a stereotaxic atlas [13].

RESULTS

NADPH-positive neurons were found on all rostro-caudal levels of the medulla, as well as in the first cervical spinal segments (Fig. 1). In particular, the most dense populations of intensively stained middle-size (15-25 μm) multipolar cells were observed in the ventral group of the reticular formation nuclei. The mean density of stained units in the nucl. paragigantocellularis lateralis reached 27.9 ± 2.6 cells per 0.1 mm² of the slice. In the nucl. reticularis rostroventralis and nucl. gigantocellularis this index was equal to 14.2 ± 2.1 and 12.7 ± 1.2 cells per 0.1 mm², respectively (Figs. 1A, B; 2A, B, and 5). This cell group could be seen up to the caudal border of the IV ventricle, and the density of positive units was highest at the level of the nucl. facialis. On more caudal levels, NADPH-d-positive neurons were observed in the ventral regions of the nucl. reticularis paramedialis neighboring the olivae inferior and in the ventral medullary reticular nucleus (on average, 10.1 ± 1.3 and 4.3 ± 0.7 cells per 0.1 mm², respectively; Figs. 1E; 3D, and 4A). In the lateral reticular nucleus there were no NADPH-d-positive cells (Fig. 1D).

A group of labeled small and medium-size neurons was also localized in the nucl. reticularis intermedialis and retrofacial region (Fig. 1A-C). We observed single small and medium-size neurons and stained fibers within the nucl. ambiguous, at all its rostro-caudal levels (the mean density of 2.9 ± 0.3 cells per 0.1 mm²); slightly stained neurons were also observed in these regions (Figs. 1B-D; 3, A, and 5). Single NADPH-d-positive neurons were as well identified at more caudal levels, within the retroambigious nucleus (Figs. 1E and 3B). Multipolar neurons (most of them were medium-sized) were found at all rostro-caudal levels of the nucl. tractus solitarii, and their highest density (the mean, 6.2 ± 0.4 cell per 0.1 mm²) was observed within the ventrolateral parts of this nucleus (Figs. 1B-E; 2C, 3C, and 5). Single labeled units were also observed in the commissure (Fig. 1E). Noticeable groupings of small positive cells were met in the nucl. prepositus hypoglossus, whereas at the caudal medullary levels (in the nucl. hypoglossus) only single stained neurons were present (Figs. 1B-E and 2D).

Clearly visible groups of labeled neurons could be traced in the peripheral parts of the raphe nuclei, along the entire dorsomedial spinal trigeminal nuclei, and caudally to the IV ventricle, in the dorsal motor vagus nucleus, and Roller's and Probst's nuclei (Fig. 1B-E).

On the level of the upper cervical spinal segments we could observe noticeable, but not very dense, accumulations of positive neurons in the layers 1-4, 7, and 10. Only sporadic positive neurons or their small groups were observed within the layers 5, 6, 8, and 9 (Figs. 1F and 4).

DISCUSSION

The central finding of our study can be described as follows: a significant number of NADPH-d-positive neurons can be observed in the medulla and adjacent regions of the spinal cord and brainstem of newborn rats. This fact allows us to suppose that within the early postnatal period, similarly to what occurs in later stages of the development, endogenous NO should be considered...