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An ultrastructural demonstration of NADPH-diaphorase/nitric oxide synthase activity in the rat striatal astroglia

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In the present study we have used electron microscopical NADPH-diaphorase (NADPH-d) histochemistry as a visualization procedure for nitric oxide synthase (NOS) to examine patterns of activity in the subcellular distribution of NADPH-d in the rat striatal astroglia. Electron microscopical examination revealed deposition of the NADPH-d reaction product in a nuclear envelope, fragments of endoplasmic reticulum and mitochondria. Predominantly mitochondria of astrocytic "end feet" were labelled. Our ultrastructural observations promote the possibility that astroglial NADPH-d/NOS is involved in adaptation of the local blood flow in the striatal microenvironment.

key words: NADPH-diaphorase, histochemistry, astrocytes, electron microscopy, nitric oxide synthase

INTRODUCTION

Astroglial cells are intimately associated with neurons and, via specialized "end feet", with cerebral blood vessels. Their ability to synthesise and release nitric oxide [4] promote the possibility that NO in astrocytes may be involved in bidirectional cell signalling which links the dynamics of the cerebral blood flow to a functional state of the neurons. In this context, the subcellular distribution of NOS in the astroglia and particularly in the perivascular "end feet" may provide essential evidence in support of this hypothesis.

MATERIAL AND METHODS

The study was carried out on 5 male Wistar rats maintained under standard conditions. The animals were perfused transcardially and tissue was fixed and processed for ultrastructural histochemistry applying the tetrazolinum salt technique for NADPH-d [1].

RESULTS AND DISCUSSION

Detailed study of the astrocytes under examination revealed that the NADPH-d reaction product was specifically associated with endomembranes of the nuclear envelope (Fig. 1, 2) comprising the entire circumference or some portion of it. Additionally, membranes of singular mitochondria and fragments of endoplasmic reticulum were labelled. Stained mitochondria were also encountered in the astrocytic processes (Fig. 1). Particularly in the "end feet" abutting blood vessels (Fig. 3) nearly half of the observed mitochondria showed BSPT formazan precipitation. Pronounced formazan deposits were also found on the luminal surface of endothelial cells (Fig. 3).

All known isoforms of NOS, including the neuronal, endothelial and inducible species, can be visualised by NADPH-d histochemistry due to the immanent NADPH-d component [5]. Astroglial cells have been found to express both constitutive and inducible NOS

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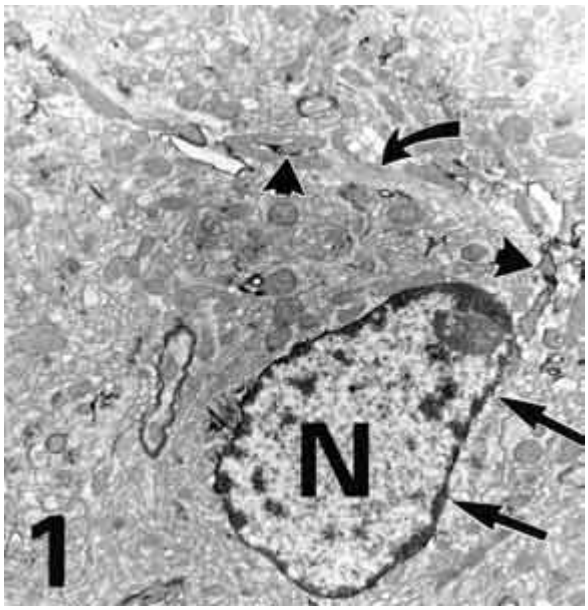
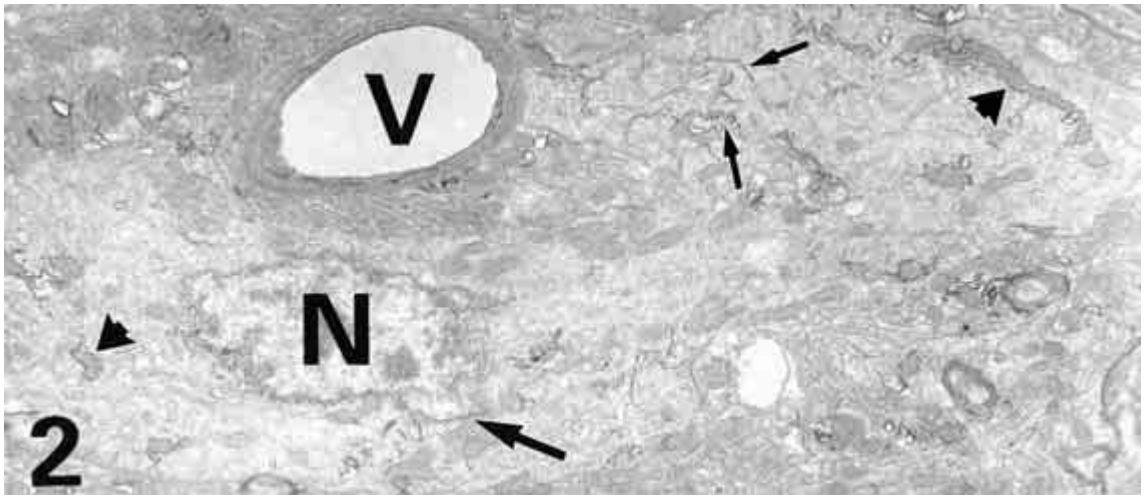
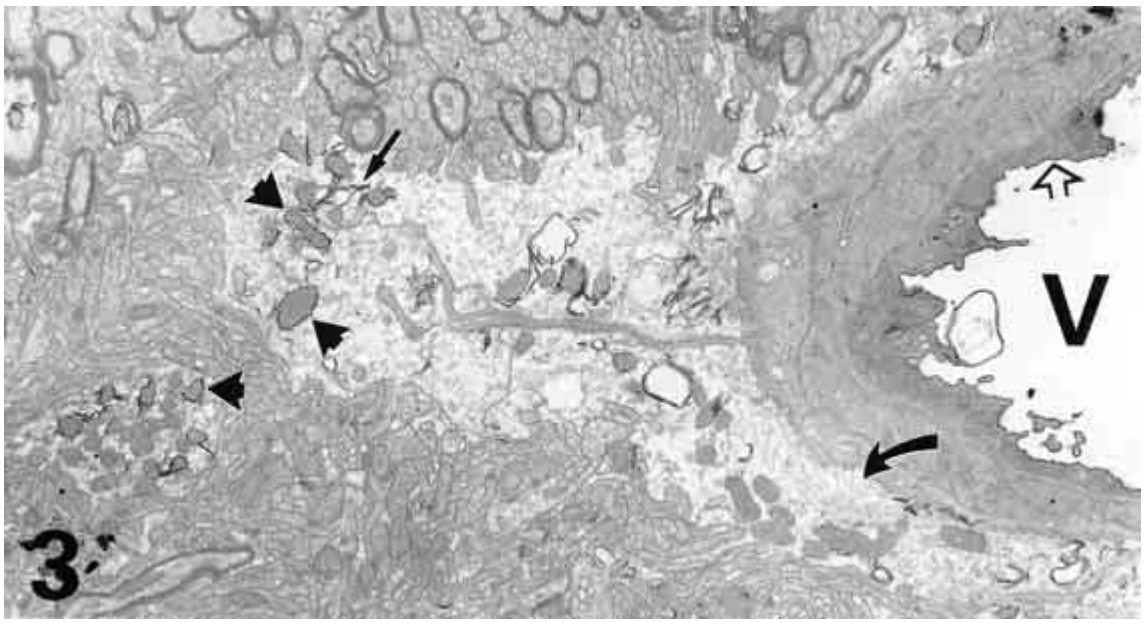


Figure 1. NADPH-d histochemical activity in a rat striatal astrocyte. Note the NADPH-d labelled membranes of the nuclear envelope (large arrows) and mitochondria (large arrowheads). The bent arrow indicates a concentration of gliofilaments; N — nucleus; × 8400.

Figure 2. NADPH-d labelled astrocyte in contact with a blood vessel. BSPT formazan is precipitated at the nuclear envelope (large arrow), mitochondria (large arrowheads) and membranes of the endoplasmic reticulum (small arrows); N — nucleus, V — blood vessel; × 8400.

Figure 3. NADPH-d activity in an astrocytic "end foot" attached to the blood vessel. Large arrowheads indicate labelled mitochondria, while the small arrow points to NADPH-d-positive endomembranes. The open arrow shows labelled endothelium. The bent arrow indicates bundles of gliofilaments; V — blood vessel; × 8400.





[6]. Our previous ultrastructural studies [1] utilizing NADPH-d histochemistry as a detection system for NOS in microglia/macrophages have shown an isoform-dependent differential localisation of the BSPT formazan at endomembranes or in cytosol for constitutive and inducible NOS respectively. Since quiescent astroglia have been found to express only constitutive NOS and as the astrocytes studied here represent unstimulated cells, it seems reasonable to propose that the NADPH-d reaction product found to be exclusively bound to membranes represents a constitutive isoform of NOS, most likely neuronal or endothelial NOS. The occurrence of NADPH-d-positive mitochondria and stained endoplasmic membrane fragments in astrocytic "end feet" apparently constitutes a NO generating machinery, indicating the potential role of NO in the regulation of local blood flow [2, 3] and, possibly, in other functions as well [6].

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