Histochemical localization of nitric oxide synthase in the CNS

In a recent article, which we enjoyed very much, Murphy *et al.*[1] reviewed the synthesis of nitric oxide (NO) in CNS glial cells. Among other results, they showed, using NADPH-diaphorase histochemistry, that nitric oxide synthase (NOS) is localized in cultured astrocytes and microglia. (Recently, it has been shown that NADPH-diaphorase is NOS and that this fully accounts for NADPH-diaphorase histochemistry[2].) However, in that article, and others on the same and related subjects[3], there has been a historical misconception, because it has never been mentioned that in the late 1950s and early 1960s histochemists had already described the NADPH-diaphorase activity not only in the brain (for example, in neurons, glial cells, cerebral blood vessels, meninges, ependyma, gliomas and other brain tumours) and in other organs (for example, in retina, heart, liver and intestines), but also in cultured rat astrocytes, oligodendrocytes, capsular satellite cells and Schwann cells[4]. Therefore the recent descriptions could be classified as a 'rediscovery'. Interestingly, the early histochemists observed NADPH-diaphorase activity in glial cell cultures that were not treated with bacterial endotoxin (lipopolysaccharide) or with combinations of cytokines while Murphy *et al.*[1] emphasized that NADPH-diaphorase activity was not apparent in untreated glial cell cultures. However, it is difficult to comment further on this difference because 'early' histochemists did not detail the intensity and incidence of NADPH-diaphorase activity in cultures and in tissues. The early histochemists described a weak staining reactivity in astrocytes in human, rat and rabbit brains[5] (see Fig. 1B), and a more intense staining reactivity in reactive and neoplastic astrocytes[5–8] and in reactive macrophages or microglia[6] or Schwann cells[9]. Murphy *et al.*[1] also stressed that the NO-producing capacity of oligodendrocytes is not yet known. Previously presented facts, and current results from our laboratory (Fig. 1A), show that oligodendrocytes and other glial cells can also express NADPH-diaphorase/NOS, and probably synthesize NO.

We suppose that one of the reasons that current investigators have failed to notice these results is that until recently the nature and function of the enzyme responsible for the NADPH-diaphorase activity remained a mystery[2]. Furthermore, the name of the enzyme has gone through several changes [for example, triphosphopyridine nucleotide (TPN)- or TPNH-cytochrome c reductase; TPNH- or NADPH-tetrazolium reductase; TPNH- or NADPH-dehydrogenase; NADPH:(acceptor) oxidoreductase; TPNH- or NADPH- or $NADPH_2$-diaphorase (E.C. 1.6.99.1.); endothelium-derived re-

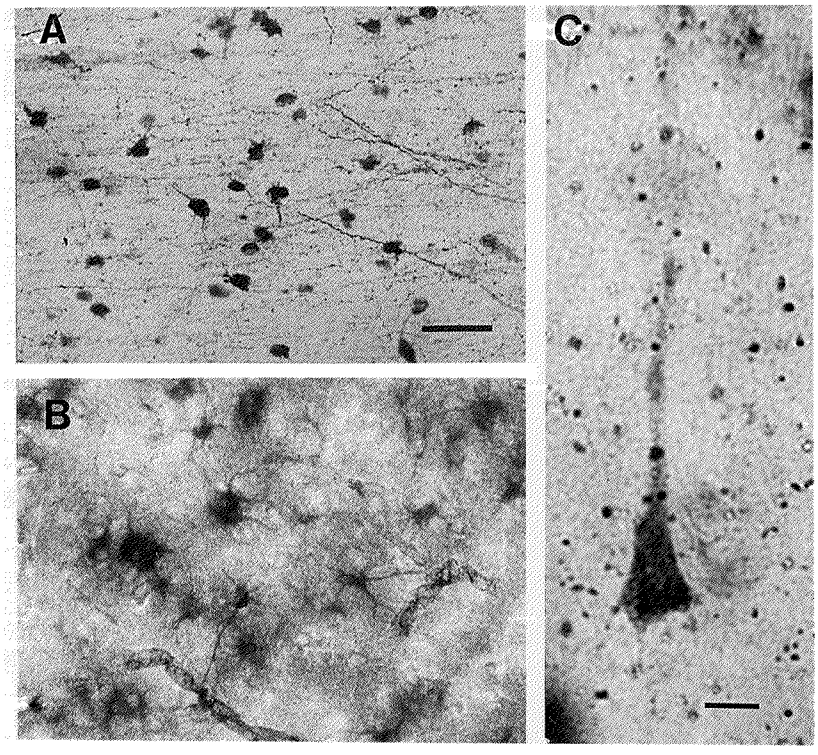

Fig. 1. *Histochemical localization of NADPH-diaphorase/nitric oxide synthase in neuronal and glial cells of the human cerebral cortex.* **(A)** *Positive oligodendrocytes in the subcortical white matter of a three-month-old infant.* **(B)** *Positive astrocytes in the pyramidal layer of the hippocampus of a three-year-old child.* **(C)** *Pyramidal neuron, with a positive cell body and proximal dendrite, in the cerebral cortex of a three-month-old infant. Scale bars, 100 μm in (A and B) and 30 μm in (C). (Šestan, N. and Kostović, I., unpublished observations.)*

laxing factor (EDRF)-synthesizing enzyme, guanylate-cyclase-activating factor (GAF) synthase and finally, NOS (E.C. 1.14.23.)], which probably caused many misunderstandings. Moreover, two different staining methods have been used; both are based on the presence of NOS, which requires NADPH as a co-factor and catalyses the NADPH-dependent reduction of dyes such as tetrazolium salts.

We suggest that this whole body of data should be viewed in the light of the evidence that NADPH-diaphorase is NOS, but it should also be cautiously and critically analysed because some of these results are incomplete and obtained by different methods. However, recent studies from our (Fig. 1B), and other[1], laboratories confirmed some of these results using both classical histochemical and modern techniques. These studies show that the distribution of NADPH-diaphorase/NOS is more widespread than has been thought, especially in the case of glia where the expression of NOS is associated with all three major types. Moreover, by showing the activity in other elements such as pyramidal neurons (Fig. 1C) in the human brain, we now know that the distribution of NOS, and its possible role, is even more widespread.

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