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Nitric oxide synthase expression in the central nervous system of *Sepia officinalis*: an *in situ* hybridization study

A. Di Cosmo[1] and C. Di Cristo[2]

[1]*Structural and Functional Biology, University of Naples 'Federico II', Naples, Italy and* [2]*Biological and Environmental Sciences, University of Sannio, Benevento, Italy*

Since the activity of the gaseous transmitter nitric oxide (NO) was first described in the gastropod mollusc *Lymnaea stagnalis*, by Winlow's group (Moroz et al. 1993) the presence and functions of NO and its synthetic enzyme nitric oxide synthase (NOS) have been investigated in bivalves, cephalopods and other gastropods. Cephalopods molluscs have the most highly developed nervous systems of all invertebrates and exhibit a number of complex behaviours; they also show a marked ability to learn. Their CNS exhibits a functional differentiation into distinct lobes. We have already described the presence of NOS rather than the presence of NADPH-diaphorase in the CNS of cephalopod *Sepia officinalis* (Di Cosmo et al. 2000). We also provided evidence for the existence of the excitatory glutamate-NO-cyclic GMP signalling pathway.

We recently reported the molecular cloning of NOS mRNA from *Sepia officinalis* (SoNOS) using a strategy involving hybridization of degenerate PCR primers to highly conserved NOS regions, combined with RACE (rapid amplification cDNA ends) procedure. Here, an *in situ* hybridization study has been performed on serial sections of the cuttlefish CNS (no. of animals = 5) to reveal specific staining of cell bodies in several lobes of the brain. Staining was found in many lower motor centres, including cells of the inferior and superior buccal lobes (feeding centres); in some higher motor centres (anterior basal and peduncle lobes); in learning centres (vertical, subvertical and superior frontal lobes); and in the visual system (medulla and deep retina (optic lobe)). Positive staining was also found in the olfactory lobe. These findings confirm our previous results on the distribution of NOS revealed by immunohistochemistry. Interestingly NOS expressing cells have been detected in the interbasal lobe, which showed no NOS immunopositivity. These data further reinforce the hypothesis that nitric oxide (NO) may be involved as a signalling molecule in feeding, motor, learning, visual and olfactory systems in the cuttlefish brain.

Di Cosmo A, Di Cristo C, Palombo A, d'Ischia M & Messenger JB (2000). J Comp Neurol 428, 411-427.

Moroz LL, Park J-H & Winlow W (1993). Neuroreport 4, 643-646.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.