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Neuronal nitric oxide synthase immunoreactivity in the respiratory tract of the frog, *Rana temporaria*

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Summary

Physiological and histochemical studies have recently supported the notion that nitric oxide (NO) is the transduction signal responsible for the non-adrenergic, non-cholinergic relaxation of the vasculature as well as the airways of the mammalian lung. We report the presence of immunoreactivity to NO synthase (NOS) in nerve cell bodies and nerve fibres in the neural plexus of the buccal cavity and lungs of the frog, *Rana temporaria*, using the indirect immunocytochemical technique of avidin–biotin and the NADPH-diaphorase technique. The neural ganglia located next to the muscle layer and within the connective tissue of the buccal cavity were partially immunoreactive for NOS. In the lungs, NOS immunoreactivity occurred in nerve cell bodies, as well as in both myelinated and unmyelinated nerve fibres. Fine nerve fibres immunoreactive to NOS were observed within the muscle fibre bundles and next to the respiratory epithelium. Both the presence of NOS immunoreactivity and the positive histochemical reaction for NADPH-diaphorase in the neural plexus of amphibian respiratory tract suggests a broad evolutionary role for NO as a peripheral neurotransmitter.

Introduction

Nitric oxide (NO) is now known to be responsible for the biological activity of endothelium-derived relaxing factor (EDRF) (Moncada *et al.*, 1991). Nitric oxide is also a novel neuronal messenger (Knowles *et al.*, 1989; Bredt *et al.*, 1990; Snyder & Bredt, 1991; Garthwaite, 1991) identified in the peripheral and central nervous system. NO is synthesized from L-arginine by a family of enzymes, the NO synthases, of which three types have been identified: the neuronal (nNOS), the endothelial (eNOS) and the immunologically-activated enzyme (iNOS). Currently there are antibodies to these isoenzymes which have been utilized for the immunohistochemical study of their distribution in many different tissues and species.

Recently, a neuronal sub-population immunoreactive to an NO synthase antibody raised against the rat brain NO synthase, has been identified both in the myenteric and respiratory neural plexi of several mammalian species including man (Belai *et al.*, 1992; Llewellyn-Smith *et al.*, 1992; Saffrey *et al.*, 1992; Ward *et al.*, 1992; Díaz de Rada *et al.*, 1993; Timmermans *et al.*, 1994). Such immunoreactivity has also been reported in the neurons of the gastrointestinal tract of amphibians, such as *Bufo marinus* (Li *et al.*, 1992, 1993; Murphy *et al.*, 1994).

The aim of the present work was to determine, by means of specific immunocytochemical techniques, whether NO synthase is also present in the neural plexus of the respiratory tract in amphibians.

Materials and methods

Six adult frogs (*Rana temporaria*) were killed by decapitation and the respiratory system dissected. The material was fixed in Bouin's fluid for 24 h, dehydrated, embedded in paraffin wax, and 3 μm-thick sections were cut. For general histological study, sections were stained with Haematoxylin and Eosin and Masson's trichrome.

Immunocytochemical technique

The presence of NO synthase, peptide histidine-isoleucine (PHI) and vasoactive intestinal polypeptide (VIP) was investigated in both random and serial adjacent paraffin sections of amphibian respiratory tract. The indirect immunocytochemical technique using avidin–biotin