Axoplasmic localisation of the NFκB p65 subunit in the rat brain

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INTRODUCTION AND METHODS. Transcription factor NFκB acts as a dimer, most frequently consisting of p50 and p65 subunits. It interacts with an inhibitory subunit, IκB protein, that inhibits its translocation from the cytoplasm to the nucleus. While NFκB involvement in mediating an inflammation is well documented, its role in regulation of other cell functions is less defined. In the nervous system the studies on NFκB focus on its role in cell survival and apoptotic death following injury. Less attention is paid to NFκB responses to physiological stimuli in the intact brain. In this respect important observation was made on localisation of p50 in the synaptosomal fraction (2). This study was followed by the report that IκB is constitutively expressed in neuronal fibres (1). Although these data point to the unique possibility of NFκB signalling operation at the non-perikaryonal level, none of the studies reported on p65 presence in fibres, a condition to form in this subcellular compartment the commonest p50/p65 dimer. Our aim was to define whether p65 is localised in neuronal processes. We undertook the immunocytochemical study using the perfusion protocol shown to improve detection of some peptides. We focused on hypothalamic nuclei basing on data indicating high perikaryonal level of p65 in this structure (1, 3). Adult male Wistar rats were anaesthetized (Nembutal, 80mg/kg, i.p.) and perfused through aorta with 0.01 M PBS, pH 7.4, followed by 2% paraformaldehyde plus 0.2% parabenzoquinone in 0.1M PB. Brains were cut at 25 μm on a cryostat. Free-floating sections were processed with rabbit polyclonal anti-p65 (A) and A(X) antibodies (0.05 - 0.02 μg/ml, Santa-Cruz). The p65 was detected with ABC Vectastain kit. Controls with antibodies preincubated with blocking peptide showed no staining.

RESULTS AND DISCUSSION. Microscopic examination revealed an intense perikaryonal and fibre staining in Zona Incerta, dorsomedial, periventricular, perifornical and lateral hypothalamic areas. A portion of p65-containing processes appeared to derive from fusiform and polygonal magnocellular (MCLH) cells (Fig.1A). Multiple fibres were passing along the optic tract (opt), while optic tract itself was devoid of staining. Our observation documents for the first time axoplasmic localisation of NFκB p65. It supports the idea of NFκB function as a retrograde messenger mediating stimulus-response coupling following synaptic stimulation.

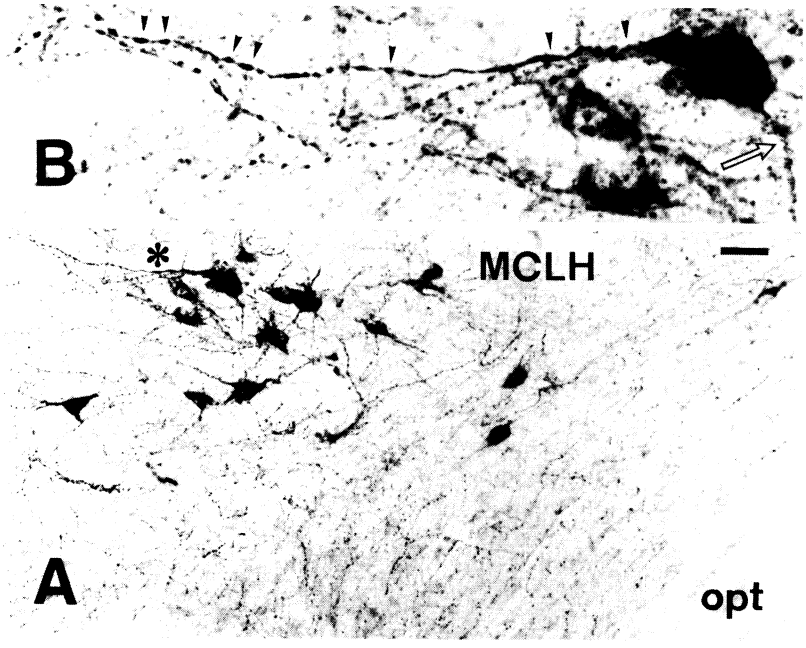

Fig. 1. Survey (A) and detail (B) of a frontal 25 μm section immunostained for the NFκB p65 protein through the lateral hypothalamic area. Arrowheads point to the axonal varicosities; an arrow points to the dendrite. Scale bar 40 μm.

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