Electron Cytochemical Localization of Gamma-Aminobutyric Acid Catabolism in Rat Cerebellar Cortex

Jonathan C. Hyde and Norman Robinson
Department of Anatomy and Histology, The London Hospital Medical College, Turner Street, London E1 2AD, England

Summary. An electron cytochemical technique is described for the localization of GABA-T, the enzyme which degrades the neurotransmitter GABA, in rat cerebellar cortex. The technique allows ultrastructural demonstration of GABA-T activity by the final deposition of an electron dense formazan precipitate at reaction sites, whilst maintaining adequate ultrastructural preservation for recognition of cellular and subcellular structures. Numerous electron dense precipitates are evident as discrete punctate deposits situated mainly in mitochondria of stellate cells, basket cells and astrocytic glial cells; they are also seen in axonal or dendritic profiles at some synaptic junctions. The technique enables the first cytochemical demonstration of the mitochondrial localization of GABA-T activity in nervous tissue to be presented. It establishes that GABA-T is present in supposed GABA neurons, in pre- or post-synaptic endings, or both, of presumed inhibitory synapses and in glial cells which may be associated with these synapses. From this seemingly ubiquitous distribution, functional aspects of GABA-T in these cells is considered.

Introduction

In the rat brain and especially cerebellar cortex, light microscopic histochemical studies have determined the distribution and localization of gamma-aminobutyric acid transaminase (GABA-T) the catabolic enzyme of the neurotransmitter GABA (Van Gelder, 1965; Kuriyama, Haber, Sisken and Roberts, 1966; Robinson and Wells, 1973; Hyde and Robinson, 1974a, b, c, d). These studies provided valuable but limited information concerning the localization of GABA degradation in brain cells and processes.

In the course of studies on GABA-T, we have developed a method for the chemical fixation of brain which allows the retention of GABA-T activity whilst maintaining good cellular morphology (Hyde et al., 1976). The method uses a low concentration mixture of formaldehyde and glutaraldehyde and has
allowed us to extend the original GABA-T histochemistry to ultrastructural levels. This paper deals with an electron cytochemical technique for GABA-T based on the precipitation of an insoluble formazan and its application to the localization of GABA degradation in brain cells of the cerebellar cortex. The work was undertaken to clarify three main points: (i) to demonstrate subcellular sites of GABA-T activity in brain cells, (ii) to establish whether GABA-T activity is restricted only to the neuronal compartment of the brain or is distributed in both neuronal and glial tissues and (iii) to determine whether GABA-T activity is present in postsynaptic sites of presumed GABA inhibitory synapses.

Materials and Methods

Fixation. Sprague-Dawley rats (250 g), lightly anaesthetized with ether, were perfused with 200 ml of cold (4°C) fixative consisting of 2% formaldehyde (prepared from paraformaldehyde) and 2% glutaraldehyde in 0.1 M phosphate buffer pH 7.4. The perfusate was introduced through the left ventricle by a gravity feed system with a head of 150 cm. The descending aorta was clamped and a small incision was made in the right atrium to allow outflow of fixative. The entire perfusion procedure was completed in less than 5 min. After perfusion cerebella were excised and cut transversely or sagittally into approximately 200 μm slices with an Oxford Vibratome sectioning system. The slices were floated off into 0.82% saline pH 7.4 containing 0.5% glutaraldehyde. The cerebellar cortex was removed from any subjacent white matter, cut into approximately 1 mm² x 200 μm blocks and incubated for GABA-T activity as below.

Histochemistry. The staining technique is a modification of a method previously described for light microscopy (Hyde et al., 1976). Tissue blocks of cerebellar cortex, about 150 in all, from 15 rats were placed directly into the GABA-T incubation medium and incubated with gentle agitation for 30-60 min at 37°C. The medium consisted of:

Tris-maleate buffer 0.1 M pH 7.6
GABA and α-ketoglutarate (disodium salt): 5 mg/ml
NAD⁺: 1 mg/ml
Nitro-blue tetrazolium (Nitro-BT): 1 mg/ml
Sodium malonate: 3 mg/ml
Sodium cyanide: 0.05 mg/ml
Phenozine methosulphate (PMS): 0.05 mg/ml
Sodium chloride: 7.2 mg/ml
Magnesium chloride: 1.02 mg/ml

In addition to these constituents, the medium also contained 0.5% Triton X-100 (Katchburian, Katchburian and Pearse, 1967).

Control tissue blocks were treated as follows:
(i) Incubated in media lacking GABA,
(ii) Preincubated for 5 min in buffer containing either 5 mg/ml amino-oxyacetic acid as GABA-T inhibitor or 1.25 mg/ml m-hydroxybenzaldehyde as SSA-DH inhibitor and then incubated in media containing the same concentrations of either inhibitor (Hyde et al., 1976).

The method depends on the oxidation of one of the transaminase reaction products, succinic semialdehyde (SSA), by the enzyme succinic semialdehyde dehydrogenase (SSA-DH), normally found coupled with GABA-T in brain tissue (Salganicoff and De Robertis, 1965). This oxidation reaction results in the reduction of the tetrazolium salt Nitro-BT to an insoluble formazan precipitate at enzyme sites.