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Rapid communication

**Parasagittal zonation of GABA-B receptors in molecular layer
of rat cerebellum**

Roger L. Albin and Sid Gilman

Department of Neurology, University of Michigan, Ann Arbor, MI 48104, U.S.A.

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Two distinct receptors mediate the effects of γ -aminobutyric acid (GABA) in the central nervous system. GABA-A receptors, which are linked to the modulatory benzodiazepine site and chloride ion channels, are the predominant mediators of inhibitory neurotransmission in the brain. GABA-B receptors are linked to potassium and calcium channels and may act by regulation of adenylate cyclase (Bowery et al., 1988). GABA-A and GABA-B receptors differ in their anatomic distributions. In the cerebellar cortex, GABA-A receptors are located mainly in the granule cell layer while GABA-B are receptors found predominantly in the molecular layer. We have found that molecular layer GABA-B receptors are distributed in parasagittal zones of high and low ligand binding. This is the first documentation of parasagittal zonation of any known receptor type in cerebellar cortex.

GABA-B receptor autoradiography was performed as previously described (Chu et al., 1987). Six male Sprague-Dawley rats (weight = 175–200 g) were decapitated and the brains were rapidly extracted from the cranial vault and frozen in crushed dry ice. Twenty micron sections were cut on a cryostat in a coronal plane and thaw mounted onto gelatin coated slides. Triplicate sections from each brain were run. Slides were prewashed in 50 mM Tris-Cl buffer (pH 7.4) containing 2.5 mM

CaCl₂ at 4°C for 30 min, and dried under a cool air stream. Slides were then immersed in ligand solution containing 20 nM [³H]GABA (Amersham, specific activity 100 Ci/mmol) in wash buffer plus 10 μ M isoguvacine to block GABA-A sites. The incubation period was 45 minutes at 4°C and nonspecific binding was assessed with addition of 100 μ M baclofen. Slides were rinsed with three quick squirts of wash buffer (at 4°C) and then one quick squirt of 2.5% glutaraldehyde in acetone and dried under a stream of hot air. Slides were apposed to tritium sensitive film (Hyperfilm, Amersham) along with known radioactive standards and exposed for five weeks. Film was developed in Kodak D-19 and analyzed with computer assisted densitometry.

All autoradiographs from all six animals showed alternating parasagittal zones of high and low [³H]GABA binding in the molecular layer of cerebellum (fig. 1). High density zones bound 540 fmol/mg protein (S.E.M. = 46.8) and the low density zones bound 361 fmol/mg protein (S.E.M. = 34.7) of [³H]GABA, a ratio of 1.5. Parasagittal zonation was marked in the vermis and less obvious in the hemispheres. The granule cell layer bound 84.7 fmol/mg protein of [³H]GABA. Further experiments will be necessary to determine if high and low [³H]GABA binding zones differ in receptor number or in affinity for [³H]GABA.

Our findings demonstrate parasagittal zonation of GABA-B receptors in the molecular layer of cerebellar cortex. Parasagittal zonation is a basic feature of cerebellar cortical architecture (Gravel

Correspondence to Roger L. Albin, Neuroscience Laboratory Building, 1103 E. Huron, Ann Arbor, MI 48104, U.S.A.

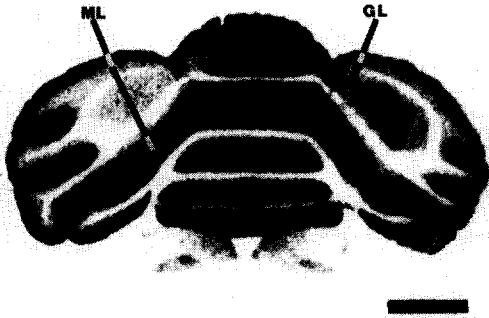


Fig. 1. Autoradiograph of GABA-B receptor distribution in rat cerebellum. High levels of binding are seen in the molecular layer. Lower density of binding is seen in the granule cell layer with little or no binding in the white matter. Parasagittal arrangement of high and low zones of [3 H]GABA binding is present in the molecular layer and is particularly evident in the vermis. ML = molecular layer. GL = granule cell layer. Scale bar = 2 mm.

et al., 1987). Both afferents and efferents of cerebellar cortex respect parasagittal zonation. Within the molecular layer, climbing fibers from subgroups of inferior olive neurons are distributed in parasagittal zones. A variety of histochemical and immunohistochemical markers are also arranged in a parasagittal fashion in cerebellar cortex (Gravel et al., 1987). As with the distribution of GABA-B receptors, the parasagittal zonation of connectional and immunohistochemical markers within the cerebellar molecular layer is most marked in the vermis. Prior studies suggest that GABA-B receptors are localized on climbing fiber terminals (Kato and Fukuda, 1985), Purkinje cell dendrites (Bowery et al., 1988) or granule cell

terminals (Wojcik and Neff, 1983). Further studies will be required to determine the relationship between GABA-B receptor zonation and other markers of parasagittal zonation within the molecular layer of cerebellum. The parasagittal zonation of GABA-B receptors suggests specialization of GABA-B receptor function within cerebellar cortex.

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