Two types of quisqualate receptors are decreased in human olivopontocerebellar atrophy cerebellar cortex

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We used receptor autoradiography to study the distribution of ionotropic and metabotropic quisqualate (QA) receptors in normal human cerebellar cortex and cerebellar cortex from 7 cases of olivopontocerebellar atrophy (OPCA). In normal human cerebellar cortex, both types of QA receptors were densest in the molecular layer. Both ionotropic and metabotropic QA receptors were significantly diminished in the molecular layer of OPCA specimens. These results suggest that both ionotropic and metabotropic QA receptors are localized on Purkinje cell dendrites.

The cerebellar cortex is a convenient region in which to study excitatory amino acid (EAA) receptors because cerebellar neuronal circuitry is relatively well understood[12,18]. Morphological and neurochemical evidence supports a neurotransmitter function for EAAs in the cerebellar cortex with glutamate as the possible neurotransmitter of mossy and parallel fibers and aspartate as the possible neurotransmitter of climbing fibers[8,11,28,30]. Electrophysiological evidence indicates that the excitatory actions of EAAs are mediated by at least 3 subtypes of ion channel-linked ('ionotropic') receptors named for the agonists which preferentially excite them: N-methyl-D-aspartate, kainate, and quisqualate[5]. In addition, a quisqualate preferring 'metabotropic' receptor linked to inositol phospholipid metabolism has been reported recently[25,26]. Receptor binding studies in animals with genetic, toxic, or viral induced depletions of cerebellar cell types and afferents have been used to determine the cellular localization of EAA receptor subtypes in the cerebellar cortex[2,4,17,21]. In particular, accumulated evidence suggests that both ionotropic and metabotropic quisqualate receptors are located on Purkinje cell dendrites[3,4,13–15,17].

We have previously reported the distribution and cellular localization of quisqualate-displaceable [^{3}H]glutamate binding in human cerebellar cortex using an assay which likely identifies but does not discriminate both the ionotropic and metabotropic quisqualate receptors[1]. In order to provide further information regarding the regional and cellular localization of quisqualate receptors in human brain, we undertook an autoradiographic study of ionotropic and metabotropic quisqualate receptors in the cerebellar cortex of neurologically normal individuals and patients with olivopontocerebellar atrophy (OPCA), a degenerative disorder characterized by degeneration of cerebellar cortical neurons, particularly Purkinje cells[9].

Blocks of cerebellar cortex were obtained at autopsy from 15 individuals and stored at −70 °C. For controls, we used 4 specimens from individuals without neurologic disease, and 4 from individuals with neurologic disease not affecting the cerebellar cortex. There was one case of multiple sclerosis, one case of Friedreich's ataxia, one case of Huntington's disease, and one case of Alzheimer's disease. The mean age at death of controls was 54 years and the mean post-mortem delay was 13.7 h. Specimens from individuals with neurologic disease not affecting the cerebellar cortex were included to control for the effects of chronic neurologic disease and motor disability. As there were no significant differences in receptor density between neurologically normal and abnormal control specimens, results from these two groups were pooled for comparison with results from the OPCA specimens. We studied 7 cases of pathologically verified OPCA. Two cases were sporadic, the remaining 5 from pedigrees with autosomal dominant inheritance. Two of the latter cases were from a well studied pedigree[24]. The mean age at death was 44.4 years and the mean post-mortem delay was 4.0 h.

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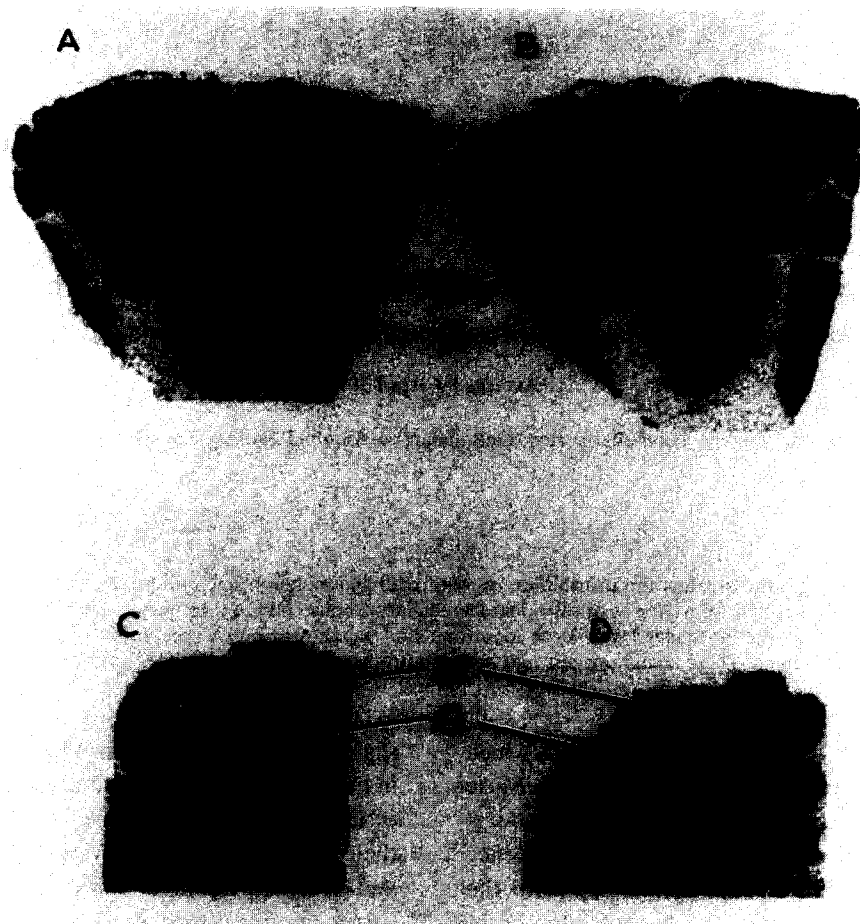
Fig. 1. Autoradiographs of [³H]glutamate binding in the presence of 10 μM AMPA and 100 μM NMDA (a measure of metabotropic quisqualate receptors) in normal human cerebellar cortex (A), OPCA cerebellar cortex (C) and [³H]AMPA binding (a measure of ionotropic quisqualate receptors) in normal human cerebellar cortex (B) and OPCA cerebellar cortex (D). Both ionotropic and metabotropic receptor densities are markedly reduced in the molecular layer of OPCA specimens and not significantly reduced in the granule cell layer. These autoradiographs represent total binding taken from two separate experiments and thus do not reflect relative receptor densities. Also, numerical data, as reported, represent total binding minus non-specific binding as stated in the text. M, molecular layer, G, granule cell layer.

Ionotropic quisqualate receptors were labelled with [³H](RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate ([³H]AMPA). We have recently described an autoradiographic method to label metabotropic quisqualate receptors by using [³H]glutamate and selective incubation conditions⁴. The binding of [³H]AMPA to ionotropic quisqualate receptors was performed according to previously published methods, and the binding of [³H]glutamate to metabotropic receptors was performed by the method of Cha et al.[4,19,20]. Briefly, 20 μm sections were cut from tissue blocks and thaw-mounted onto gelatin-coated slides. Slides for both assays were pre-washed for 30 min at 4 °C in buffer (50 mM Tris-HCl + 2.5 mM CaCl₂ + 100 mM KSCN, pH 7.2) and then dried under a stream of cool air. Slides were immersed for 45 min in incubation buffer plus 37 nM [³H]AMPA (spec. act. 29.2 Ci/mmol; New England Nuclear) to label ionotropic receptors, or 200 nM [³H]glutamate (spec. act.

46 Ci/mmol; Amersham) in the presence of 100 μM N-methyl-D-aspartate and 10 μM AMPA to label metabotropic receptors. For [³H]AMPA binding, non-specific

TABLE I

Comparison of ionotropic and metabotropic quisqualate receptor density in the molecular layer and granule cell layer in control human and olivopontocerebellar atrophy (OPCA) cerebellar cortex

All values in pmol/mg protein (mean ± S.E.M.).

	Control	OPCA	% Change
Ionotropic quisqualate receptors ([³H]AMPA)			
Molecular layer	1.02 ± 0.07	0.51 ± 0.05	−50.2*
Granule cell layer	0.25 ± 0.02	0.20 ± 0.02	−18.7
Metabotropic quisqualate receptors (AiQsGB)			
Molecular layer	0.14 ± 0.02	0.04 ± 0.01	−67.9*
Granule cell layer	0.04 ± 0.01	0.02 ± 0.01	−44.7

*P < 0.01 by the Mann-Whitney U-test.

binding was determined in the presence of 1 mM unlabelled glutamate and represented less than 5% of total binding. For [^{3}H]glutamate binding to metabotropic receptors, non-specific binding was determined in the presence of 2.5 μM quisqualate and represented approximately 45% of total binding. This AMPA-insensitive, quisqualate-sensitive glutamate binding (AiQsGB) has a pharmacological profile consistent with that of the metabotropic EAA receptor and is distinct from that of the AMPA-sensitive ionotropic receptor[4]. After incubation, sections were rinsed 3 times with cold buffer and once with 2.5% (v/v) glutaraldehyde in acetone and rapidly dried under a stream of warm air (total rinse time less than 10 s). Sections were placed in X-ray cassettes along with known radioactive standards, apposed to tritium-sensitive film (Hyperfilm, Amersham, Corp.), exposed for 2 weeks at 4 °C and developed in D-19 (Kodak). Autoradiographic images were analyzed by computer-assisted densitometry (Imaging Research, St. Catherines, Ont., Canada). The significance of differences between control and OPCA specimens was assessed with the Mann–Whitney U-test. In addition, adjacent sections were fixed over paraformaldehyde vapor and stained with Cresyl violet.

In the molecular layer of OPCA cerebellar cortex, mean specific [^{3}H]AMPA binding was significantly reduced to 50% of the control values ($P < 0.01$) but was not significantly altered in the granule cell layer (Table I, Fig. 1). AiQsGB was reduced in the molecular layer of OPCA cerebellar cortex to 32% of the control values ($P < 0.01$) and there was a non-significant trend towards reduction in the granule cell layer (Table I, Fig. 1). Cresyl violet-stained sections showed a marked loss of Purkinje cells, marked thinning of the molecular layer, and diminution of the granule cell layer.

Although our single point analysis cannot differentiate between a decrease in receptor number or affinity, Tsiotos et al.[27] have reported saturation studies of [^{3}H]glutamate binding in some of the same OPCA cases used in the present study and found that the decrease in binding represents a change in receptor number and not in the affinity of the glutamate binding sites. However, the [^{3}H]glutamate binding assay used by Tsiotos et al.[27] does not discriminate between ionotropic and metabotropic quisqualate receptors. It has been suggested that ionotropic and metabotropic quisqualate receptors are localized on Purkinje cell dendrites[3,4,17]. The OPCA cases analyzed in this study exhibited a marked reduction of Purkinje cells and in previous studies of Purkinje cell deficient *nervous* mutant mice we have found a similar reduction in [^{3}H]AMPA binding[17]. The large decrease in [^{3}H]AMPA binding in the molecular layer of OPCA cerebellar cortex supports the hypothesis that ionotropic

quisqualate receptors are located on the dendrites of Purkinje neurons in human cerebellum. Our finding of decreased density of AiQsGB in OPCA cerebellar cortex is also similar to studies of Purkinje cell deficient *nervous* mutant mice[4]. It has recently been hypothesized in rodents that the excitatory amino acid-linked inositol phospholipid second messenger system is most abundant in the cerebellum[10]. With antibodies directed against the IP$_3$ receptor, the cerebellar cortical inositol phospholipid second messenger system has been localized to Purkinje cell dendrites[23]. Furthermore, the highest densities of both [^{3}H]IP$_3$ binding and AiQsGB have been reported to be in the molecular layer of cerebellum where Purkinje cell dendrites are located[4,29]. Our finding of a decrease in binding to metabotropic receptors in OPCA cerebellar cortex complements a recent report by Kish et al.[16] of diminished [^{3}H]inositol 1,4,5-triphosphate ([^{3}H]IP$_3$) binding in OPCA cortical homogenates. Kish et al.[16] inferred from their OPCA data that the IP$_3$ receptor system is localized to human Purkinje cells and our OPCA data suggest that the inositol phospholipid-linked quisqualate receptor is also localized to human Purkinje cell dendrites.

The present study represents the first autoradiographic analysis of both ionotropic and metabotropic quisqualate receptors in normal human and OPCA cerebellar cortex. Our findings are concordant with prior experimental animal data[4,17,20]. In particular, binding to metabotropic and ionotropic quisqualate receptors in the *nervous* mutant mouse, which exhibits a marked loss of Purkinje cells, reveals binding characteristics similar to those obtained in our OPCA cases. Earlier anatomical studies have established that the disappearance of cerebellar Purkinje cells is a consistent feature of OPCA. Although we cannot exclude the possibility that these binding sites are located on climbing fibers or parallel fiber terminals which are also diminished in OPCA, our results, in conjunction with animal experimental data, suggest that in the human cerebellar cortex both ionotropic and metabotropic quisqualate receptors are concentrated on Purkinje cell dendrites. A glutamate neurotoxic hypothesis has been proposed for the pathogenesis of OPCA[22], and both ionotropic and metabotropic quisqualate receptors have been implicated in neurotoxic processes[6,7]. While the etiologic significance of our findings is unclear, enrichment of both subtypes of quisqualate receptors on Purkinje cells could render these cells vulnerable to EAA neurotoxicity in OPCA.

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