Autoradiographic localization of inhibitory and excitatory amino acid neurotransmitter receptors in human normal and olivopontocerebellar atrophy cerebellar cortex

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We used standard techniques of receptor autoradiography to study the distribution of inhibitory and excitatory amino acid neurotransmitter receptors in human normal cerebellar cortex. Benzodiazepine (BDZ) receptor density was relatively high in both granule cell and molecular layers. GABA$_A$ receptor density was highest in granule cell layer with lower receptor density in molecular layer. There was a lower density of GABA$_B$ receptors than GABA$_A$ receptors in both molecular and granule cell layers with a relatively higher density of GABA$_B$ receptors in molecular layer than in granule cell layer. In granule cell layer, the density of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors was greatest whereas in molecular layer the quisqualate (QA) receptor subtype density was greatest. With [³H]N-(1-[2-thienyl]cyclohexyl)3-4-piperidine as a ligand, there was no specific binding to the phencyclidine receptor. Molecular layer was also characterized by relatively high density of a non-NMDA/non-QA displaceable glutamate binding site. We studied also the cerebellar cortex of 4 cases of olivopontocerebellar atrophy (OPCA), a syndrome in which Purkinje and granule cells degenerate. In these specimens, there was significant decrement of BDZ and GABA$_A$ receptors in both molecular and granule cell layers, with loss of GABA$_B$ receptors in molecular layer. NMDA receptors were depleted in granule cell layer while QA receptors and the non-NMDA/non-QA glutamate binding site were significantly depleted in molecular layer. Our normal human and OPCA data are largely consistent with animal data about the cellular localization of cerebellar cortical amino acid neurotransmitter receptors.

INTRODUCTION

The relatively simple architecture and uniform organization of the cerebellar cortex have made it a favored area for the study of synaptic physiology and neurotransmitter action[21]. Excitatory amino acids and gamma aminobutyric acid (GABA), the primary excitatory and inhibitory neurotransmitters of the central nervous system, are thought currently to mediate most synaptic events within the cerebellar cortex. The excitatory amino acid glutamate (GLU) is probably the neurotransmitter of mossy fibers while the closely related excitatory amino acid aspartate may be the neurotransmitter of climbing fibers[3,16,17,41,47,50,57]. Granule cells appear to use GLU as their neurotransmitter[21,43,49,61] while Purkinje cells, stellate cells, basket cells, and Golgi cells are thought to be GABAergic[22,28,36,59].

Both excitatory amino acid and GABA receptors possess subtypes. Two general types of GABA receptor are recognized. GABA$_A$ receptors mediate inhibition through a chloride ion channel modulated by the linked benzodiazepine (BDZ) receptor[14]. GABA$_B$ receptors

modulate potassium or calcium channels through second messenger systems[6]. Excitatory amino acid receptors have been divided into 3 categories, N-methyl-D-aspartate (NMDA), quisqualate (QA), and kainate receptors, based on studies of these relatively selective agonists[33]. Associated with the NMDA receptor ionophore is the phencyclidine (PCP) receptor, the binding site for PCP and other dissociative anesthetics[33]. Receptor binding techniques, particularly receptor autoradiography (ARG), have been used to define the anatomic distribution of excitatory amino acid and GABA receptors within the cerebellar cortex of experimental animals[7,12,18,34,35,37-39,42,62]. Application of receptor binding techniques to animals with genetic, toxin, or viral induced degeneration of specific cerebellar cortical neuron subpopulations and afferents has given valuable information about the cellular localization of different neurotransmitter receptors within cerebellar cortex[2,8-11,23,27,31,32,34,35,42,45,46,48,58].

In this report, we describe the distribution of GABA$_A$, GABA$_B$, BDZ, NMDA, QA, and PCP receptors in normal human cerebellar cortex as determined by ARG.

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In addition, we studied the distribution of these receptors in cerebellar cortical specimens from individuals suffering from olivopontocerebellar atrophy (OPCA), a clinically and genetically heterogeneous syndrome in which degeneration of cerebellar cortical neurons, particularly Purkinje and granule cells, and loss of climbing fiber and mossy fiber afferents are constant features[4,19]. The use of OPCA specimens is comparable to the use of rodents with genetic, viral, or toxin induced loss of cerebellar cortical neuron subpopulations and afferents and permits inferences about the cellular localization of these receptors. A preliminary account of this work has appeared[1].

MATERIALS AND METHODS

Materials

[3H]GABA, [3H]flunitrazepam, and [3H]GLU were purchased from Amersham (Arlington Heights, IL). [3H]N-(1-[2-thienyl]-cyclohexyl)3-4-piperidine (TCP) was purchased from NEN-Dupont (Boston, MA). Non-radioactive NMDA and QA were purchased from Cambridge Research Biochemicals (Valley Stream, NY). Non-radioactive PCP was a generous gift from Dr. James H. Woods. Tritium sensitive film was purchased from LKB (Ultrofilm; Piscataway, NJ). The remaining reagents were purchased from various commercial suppliers and were of the highest possible purity.

Human specimens

Blocks of cerebellar cortex were obtained at autopsy from 12 individuals and then stored at -70 °C. We studied 4 cases of pathologically verified OPCA. One case was sporadic, and the remaining 3 were from pedigrees with autosomal dominant inheritance. Two of the latter cases were from a well studied pedigree[44]. The mean age at death was 38 years (range 15 to 72). The mean postmortem delay from autopsy to freezing was 3.25 h (see Tsiotos et al. for a more extensive description of these cases[52]). 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 of Friedreich's ataxia, one of Huntington's disease, and one of Alzheimer's disease. The mean age at death was 54 years (range 23 to 87), and the mean postmortem 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.

Autoradiography

Tissue blocks were warmed overnight to -20 °C and sectioned at 20 μm on a Lipshaw cryostat. Sections were thaw-mounted onto gelatin-coated slides. Slides were stored at -20 °C until the time of assay. For GLU receptors, all experiments were performed within 48 h of sectioning. All GABA receptor assays were done within 72 h of sectioning, and BDZ and PCP receptors were assayed within 5 days of sectioning. All ARG assays followed the same basic procedure. Slides were prewashed in incubation buffer at 4 °C, air-dried under a stream of cool air, and then immersed in incubation buffer at 4 °C with tritiated ligand and appropriate blocking agents. Following incubation with ligand solutions the slides were rinsed and dried under a stream of hot air. Slides were apposed to tritium sensitive film along with known radioactive standards and stored for varying lengths of time in light tight cassettes. Films were developed in Kodak D-19 and analyzed using the MCID image processing system (Imaging Research, St. Catherines, Ontario). Film density was converted into bound radioactivity with a polynomial regression curve derived from the standards. All

slides were run in duplicate and at least 50 readings were taken of each area. Molecular and granule cell layers were readily identified in all specimens, but the Purkinje cell layer was not consistently identifiable. Measurements of receptor density were confined consequently to granule cell and molecular layers. Receptor density was assessed at a single concentration of tritiated ligand in each assay. All receptor assays have been described previously and used for analysis of human postmortem tissue[13,29,54,60].

For assay of BDZ receptors, slides were prewashed in 50 mM Tris-citrate buffer (pH 7.0) for 30 min, then incubated for 30 min in the same buffer containing 5 nM [3H]flunitrazepam (specific activity = 85 Ci/mmol). Slides then received one quick dip in buffer, followed by two 5-min washes. Non-specific binding was assessed by addition of 2 μM clonazepam.

For assay of PCP receptors, slides were prewashed in 50 mM Tris-acetate buffer (pH 7.4) for 30 min, then incubated for 45 min in the same buffer plus 1 mM Mg-acetate containing 20 nM [3H]TCP (specific activity = 60 Ci/mmol). Slides then received three 1-min washes in Tris-acetate plus Mg-acetate buffer. Non-specific binding was assessed by addition of 20 μM PCP.

GABA receptors were assayed with 20 nM [3H]GABA (specific activity = 105 Ci/mmol). Slides were prewashed for 30 min in 50 mM Tris-HCl plus 2.5 mM $CaCl_2$ (pH 7.4), then incubated in the same buffer containing radioactive ligand. $GABA_A$ receptors were selectively assayed by adding 100 μM baclofen, and $GABA_B$ receptors by addition of 10 μM isoguvacine. Non-specific binding was assessed by addition of both 100 μM baclofen and 100 μM isoguvacine. After incubation in the ligand solution, slides were rinsed with 3 rapid squirts of buffer followed by one squirt of 2.5% glutaraldehyde in acetone.

NMDA and QA receptors were assayed with 20 nM [3H]GLU (specific activity = 48–53 Ci/mmol). QA receptor density was determined by subtracting from total [3H]GLU binding the amount displaced by 2.5 μM QA when [3H]GLU binding was examined in 50 mM Tris-HCl plus 2.5 mM $CaCl_2$ buffer (pH 7.2). NMDA receptor density was assayed in 50 mM Tris-acetate buffer (pH 7.2) plus 2.5 μM QA. For NMDA binding, non-specific binding was assessed by addition of 1 mM GLU. In previous studies of rat and postmortem human brain we found these two assays to be the best

TABLE I

Neurotransmitter receptor density in control human and olivopontocerebellar cerebellar cortex (OPCA)

All values in fmol/mg prot. (mean ± S.E.M.).

Receptors	Control	OPCA	% Change
Benzodiazepine			
Granule cell layer	482 ± 28	163 ± 27	−65*
Molecular layer	521 ± 42	319 ± 5	−39**
$GABA_A$			
Granule cell layer	1195 ± 47	804 ± 91	−37*
Molecular layer	365 ± 28	291 ± 25	−20**
$GABA_B$			
Granule cell layer	35 ± 8	15 ± 5	−57
Molecular layer	125 ± 25	52 ± 22	−58**
Quisqualate			
Granule cell layer	65 ± 21	31 ± 4	−52
Molecular layer	109 ± 16	49 ± 8	−55*
NMDA			
Granule cell layer	53 ± 13	15 ± 7	−72**
Molecular layer	16 ± 5	13 ± 3	−19
Non-NMDA/Non-QA binding site			
Granule cell layer	12 ± 4	11 ± 6	−8
Molecular layer	34 ± 7	9 ± 3	−74**

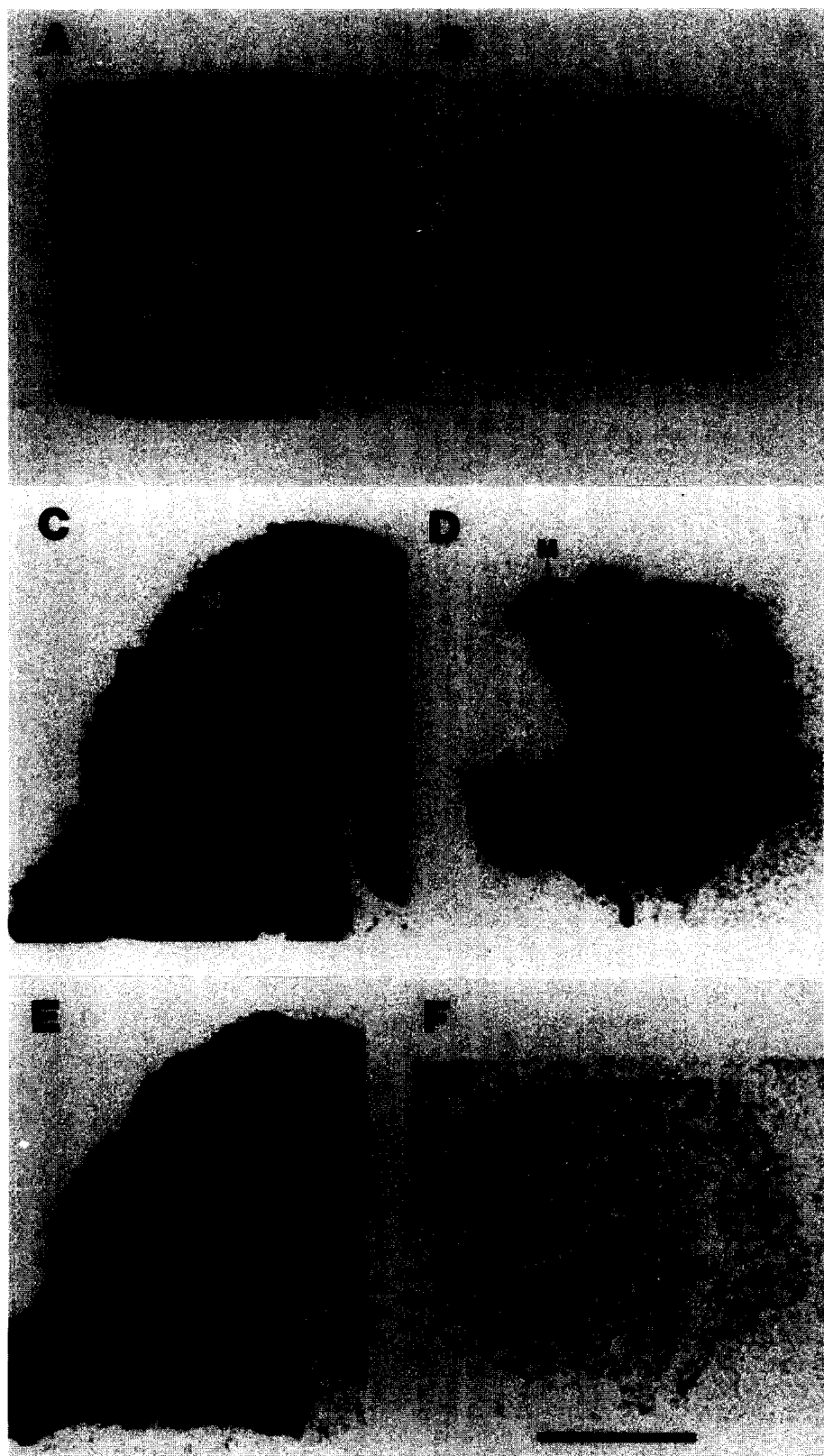*$P < 0.025$; **$P < 0.05$ (Mann–Whitney U test).

Fig. 1. Autoradiographs of benzodiazepine (A + B), $GABA_A$ (C + D), and $GABA_B$ (E + F) receptors in normal human (A,C,E) and olivopontocerebellar atrophy (B,D,F). The marked atrophy typical of these olivopontocerebellar atrophy specimens is evident in autoradiographs B,D,F. BDZ and $GABA_A$ receptor density is decreased in OPCA granule cell and molecular layers, $GABA_B$ receptor density is decreased in the molecular layer. M, molecular layer; G, granule cell layer. Scale bar = 5 mm.
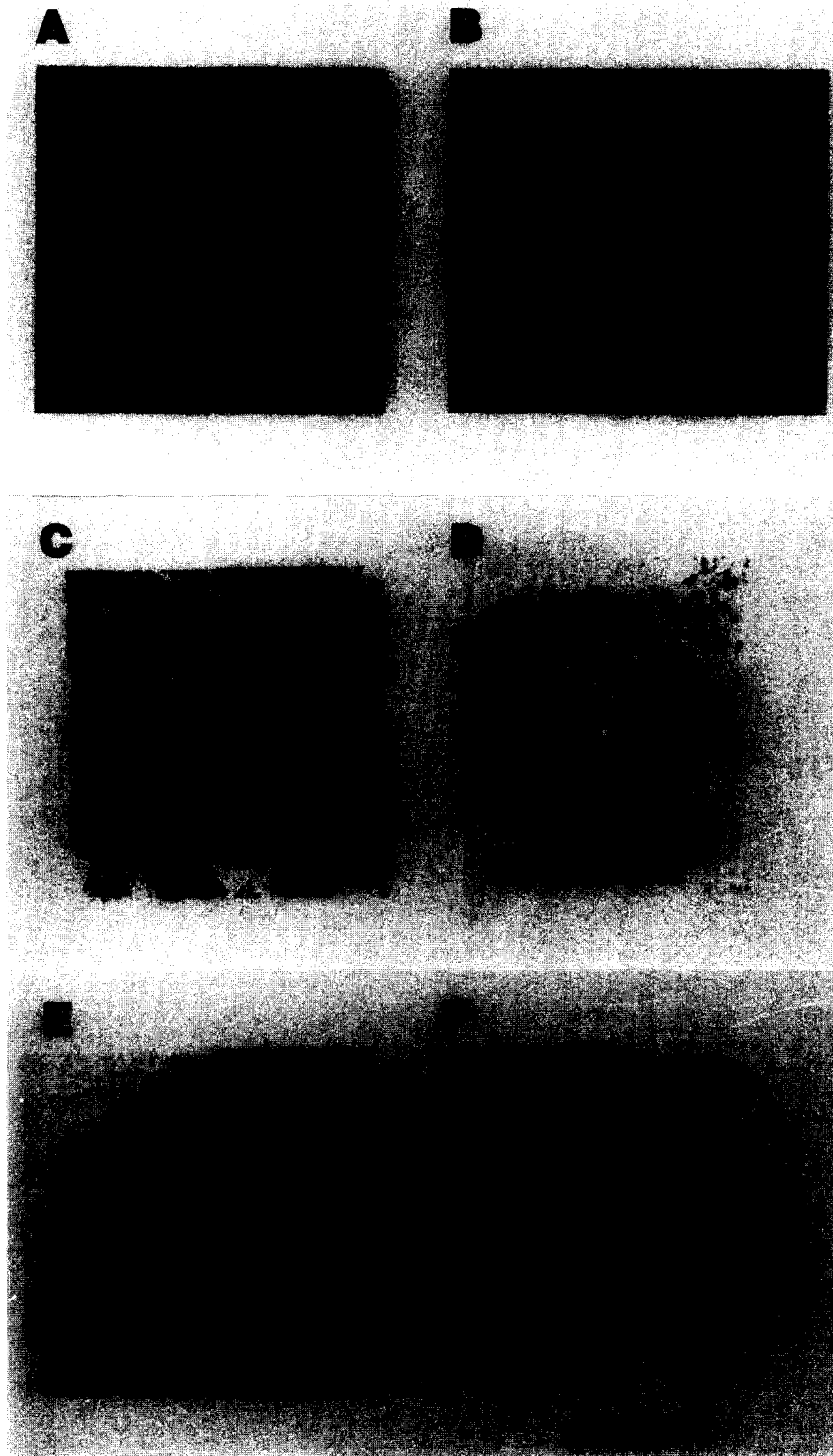
Fig. 2. Autoradiographs of quisqualate receptors (A + B), NMDA receptors (C + D), and non-NMDA/non-QA (E + F) binding in normal human (A,C,E) and olivopontocerebellar atrophy (B,D,F). Quisqualate receptor density and non-NMDA/non-QA binding are reduced in the molecular layer while NMDA receptor density is reduced in the granule cell layer of the OPCA specimens. The quisqualate receptor images are produced by digital subtraction of an image of [³H]GLU binding in the presence of 2.5 µM quisqualate from an image of total [³H]GLU binding to produce a visual analogue of the method used to determine quisqualate sensitive binding. M, molecular layer; G, granule cell layer. Scale bar = 5 mm.

means of measuring QA and NMDA receptors[18,60]. When [3H]-GLU binding was performed in 50 mM Tris-HCl plus 2.5 mM CaCl2 we determined also the amount of specific binding left after the inclusion of 2.5 μM QA and 100 μM NMDA. Non-specific binding for the non-NMDA/non-QA displaceable binding was determined by the inclusion of 1 mM GLU. After a 30-min prewash in buffer, slides were incubated in buffer plus ligand with appropriate blockers for 45 min. Slides then received 4 quick squirts with buffer, followed by 3 quick squirts with 2.5% glutaraldehyde in acetone.

Data analysis

As there were no significant differences in receptor density between the neurologically normal and abnormal control specimens, results from these two groups were pooled for comparison with results from the OPCA specimens. Differences in ligand binding between control and OPCA specimens are assumed to reflect changes in receptor density rather than changes in receptor affinity. This assumption is supported by prior homogenate studies. Kish et al.[26] found no difference in the K_d of BDZ receptors between OPCA and control specimens. Tsiotos et al.[52] compared total [3H]GLU binding in OPCA and control cerebellar cortex and found no differences in K_d or pharmacologic responses between OPCA and control specimens. The significance of differences in ligand binding between OPCA and control specimens was assessed with the Mann–Whitney U test.

Histology

Sections adjacent to those used for ARG were fixed over formaldehyde vapor and stained with 0.5% Cresyl violet. Sections were dehydrated in graded alcohols and xylene and coverslips were affixed with Permount.

RESULTS

Histology

All OPCA specimens had almost complete loss of Purkinje cells, marked thinning of molecular layers and diminution of granule cell layers. The morphology of all control specimens appeared normal, including specimens from individuals with neurologic disease not affecting the cerebellum.

BDZ ARG

In control specimens, both molecular and granule cell layers exhibited high densities of BDZ receptors (Table I, Fig. 1). In OPCA specimens, there was a marked reduction in BDZ receptor density in both granule cell and molecular layers (Table I, Fig. 1).

GABA ARG

The overall density of GABA_A receptors was significantly higher than GABA_B receptors (Table I, Fig. 1). In control specimens, GABA_A and GABA_B receptors were distributed differentially within cerebellar cortex. GABA_A receptor density was highest in granule cell layer, while GABA_B receptor density was highest in molecular layer (Table I, Fig. 1). In OPCA specimens, GABA_A receptor density was reduced significantly in both molecular and granule cell layers (Table I, Fig. 1). GABA_B receptor density was reduced significantly in molecular

layer and there was a non-significant trend towards reduction in granule cell layer (Table I, Fig. 2).

GLU ARG

QA and NMDA receptors were distributed differentially in the normal human cerebellar cortex (Table I, Fig. 2). QA receptor density was highest in the molecular layer with NMDA receptor density greatest in the granule cell layer. When [3H]GLU binding was measured in 50 mM Tris-HCl plus 2.5 mM CaCl2 with the addition of 2.5 μM QA and 100 μM NMDA, there was an additional specific [3H]GLU binding site (Table I, Fig. 2). This non-NMDA/non-QA binding site was present in both molecular and granule cell layers with greatest density in the molecular layer. Significant decreases in all 3 [3H]GLU binding sites were found in OPCA specimens. QA receptor density was reduced in the molecular layer with a non-significant trend towards reduction in the granule cell layer, NMDA receptor density was diminished in the granule cell layer, and non-QA/non-NMDA [3H]GLU binding was reduced in the molecular layer (Table I, Fig. 2).

PCP ARG

There was no specific binding of [3H]TCP in cerebellar cortex (data not shown).

DISCUSSION

Our results indicate that the distribution of inhibitory and excitatory amino acid neurotransmitter receptors in human cerebellar cortex is qualitatively similar to the distributions of receptors described in other mammals[7,12,30,35,37–39,62]. The sole exception seems to be BDZ binding. In our material and previous studies of normal human cerebellar cortex, both granule cell and molecular layers are richly endowed with BDZ receptors[15,55,61]. In contrast, rat cerebellar cortex is distinguished by a high density of BDZ receptors only in the molecular layer[38,61]. Given the overall similarity in the distribution of receptor sites, it is likely that the cellular localizations of inhibitory and excitatory neurotransmitter receptors are the same as those described in other mammals. This latter inference is buttressed by the results we obtained with OPCA specimens.

Rodents with genetic or toxin induced loss of Purkinje cells or Purkinje cell dendrites exhibit diminished molecular layer density of BDZ, GABA_A, and QA receptors[2,8,11,27,32,35,46,48]. Animals with 3-acetylpyridine induced degeneration of climbing fibers have normal density of BDZ and GABA_A receptors[8,10,23]. Similarly, genetic, toxin or viral induced loss of granule cells results in depletion of granule cell layer GABA_A and NMDA

receptors[11,31,34,42,45]. These results suggest that in rodent molecular layer, BDZ, GABA$_A$, and QA receptors are located primarily on Purkinje cell dendrites; and that in granule cell layer, GABA$_A$ and NMDA receptors are located primarily on granule cells. Our data from OPCA cerebellar cortex yield similar results. Purkinje cells were decreased markedly in all OPCA specimens and granule cells were clearly depleted as well. Consistent with the rodent data, we observed substantial reductions in BDZ, GABA$_A$, and QA receptor density in the molecular layer of cerebellar cortex in OPCA. NMDA and GABA$_A$ receptor density was diminished in the granule cell layer of cerebellar cortex in OPCA. Like rodents, molecular layer BDZ, GABA$_A$ and QA receptors are probably on Purkinje dendrites while granule cell layer GABA$_A$ and NMDA receptors are located on granule cells in humans. Because OPCA is characterized also by loss of climbing and mossy fibers, we cannot exclude the possibility that some receptors are located on these cerebellar cortical afferents, rather than on intrinsic cerebellar cortical neurons.

For GABA$_B$ receptors, the picture appears somewhat more complex, with evidence from rodent studies suggesting localization of receptors on Purkinje cell dendrites, climbing fibers, and parallel fibers[8,9,23,58]. Our results in OPCA cerebellar cortex are consistent with all these cellular localizations as our specimens exhibited both Purkinje cell and granule cell loss. Climbing fiber loss also occurs regularly in OPCA.

In OPCA specimens, we found reductions in granule cell layer BDZ receptor density, and in molecular layer the density of the non-NMDA/non-QA [^{3}H]GLU binding site was reduced. The most parsimonious explanation for these results would be localization of some BDZ receptors to granule cells and localization of the non-NMDA/non-QA site to Purkinje cell dendrites. Again, we cannot exclude the possibility that granule cell layer BDZ receptors and the non-NMDA/non-QA [^{3}H]GLU binding site are located on afferent terminals. As OPCA results frequently in loss of climbing fibers and mossy fibers in addition to Purkinje cell and granule cell depletion, the granule cell layer BDZ receptors could be located on mossy fiber terminals and the non-NMDA/non-QA [^{3}H]GLU binding site on climbing fiber and/or parallel fiber terminals.

Our finding of a non-NMDA/non-QA displaceable [^{3}H]GLU binding site is not unique. Since these assay conditions inhibit binding to the kainate receptor, it is unlikely that this binding site represents a population of kainate receptors[18,20]. Recent studies in our laboratory have documented the existence of this site in rat[20]. Lesion studies in rats have shown that this site is neuronal, a conclusion supported by our present discovery of de-

creased density of this site in cerebellar cortex of OPCA patients. In rat, however, there is little binding to this site in the adult cerebellum[20].

Our data reveal differences in the distribution of receptors thought to be functionally linked. BDZ receptors are thought to be part of the GABA$_A$ receptor complex and are found usually in association with GABA$_A$ receptors[14,55]. We observed a high density of BDZ receptors but a relatively low density of GABA$_A$ receptors in the molecular layer. A similar pattern has been observed in rodent cerebellar cortex[55]. Similarly, using [^{3}H]TCP we found no specific binding to the PCP receptor in human cerebellar cortex despite the presence of NMDA receptors. This discrepancy has been noted previously in rat cerebellum where a disproportionately low level of [^{3}H]TCP binding has been found despite the presence of a moderate density of NMDA receptors[30]. The PCP receptor appears to exist in high and low affinity forms for binding to TCP, with the low affinity form predominating in the cerebellum[53]. Nonetheless, [^{3}H]-TCP binding is seen in rat granule cell layer[30,53]. Our control human data suggest that the human cerebellar cortical PCP receptor may have a particularly low affinity for TCP.

Our findings in OPCA specimens are at variance with previous studies of BDZ and GABA receptors in this syndrome. Kish et al. have reported normal levels of BDZ receptors and increased levels of GABA$_A$ receptors in homogenates of cerebellar cortex from OPCA patients[25,26]. Whitehouse et al. used receptor autoradiography to examine 4 cases of OPCA and reported diminished [^{3}H]muscimol binding to GABA$_A$ receptors in the granule cell layer and normal binding of [^{3}H]-flunitrazepam in both layers[56]. The discrepancies between our results and prior work may be explainable in part on the basis of differences in assay technique, i.e., homogenate binding versus ARG, or by the choice of ligands. [^{3}H]Muscimol, for example, binds to a high affinity form of the GABA$_A$ receptor[54]. [^{3}H]GABA may be a less selective ligand in this respect. We have observed [^{3}H]GABA binding in the dentate nucleus (Albin et al., unpublished data), an area where [^{3}H]-muscimol does not bind[56].

Another explanation for the difference between our results and prior work lies in the nature of OPCA material. OPCA is a clinically, genetically, and pathologically variable syndrome and the degree of Purkinje cell and granule cell loss is also variable. All the specimens we studied had marked loss of Purkinje cells and loss of granule cells. Based on the published illustrations, it appears that the specimens studied by Whitehouse et al. had considerably less loss of both cell types. Kish et al. used a large number of specimens with

a variable degree of cell loss as judged by neurochemical assays[25,26]. Cases with mild to moderate cell loss might not show the changes in receptor density exhibited by our severely affected material, especially as atrophy of the cerebellar folia might elevate receptor density and obscure negative changes in absolute receptor number in specimens with mild to moderate cell loss. Our cases, with their marked Purkinje cell loss and granule cell depletion, are more similar to the various animal models of cerebellar neuron depletion than OPCA cases with mild to moderate loss of Purkinje and granule cells. Kish et al. have also suggested that early OPCA might be marked by upregulation of GABA$_A$ receptors on the remaining cerebellar neurons[25]. Although there is no direct evidence to support this hypothesis, Troncoso et al. have observed increased density of cerebellar cortical BDZ receptors in the early stages of canine inherited ataxia, a disorder of Gordon setters characterized by gradual degeneration of Purkinje and granule cells[51].

Our results extend the work of Tsiotos et al.[52], who recently described decreased [^{3}H]glutamate binding sites in homogenates from the same OPCA cases studied in the present report. Tsiotos et al. did not measure glutamate receptor subtypes and the use of homogenates precluded regional localization of decreases in receptor subtypes. Nonetheless, the present data, obtained with different assays of GLU receptors, reveal changes of similar magnitude to those reported by Tsiotos et al.

Our finding of decreased density of QA receptors in the molecular layer complements a recent report by Kish et al. of diminished [^{3}H]inositol 1,4,5-triphosphate binding in OPCA cerebellar cortical homogenates[24]. In rodent cerebellum, the phosphatidylinositide (PPI) second messenger system is associated with Purkinje cell dendrites[5]. Kish et al. inferred from their OPCA data a similar localization in human cerebellar cortex. The PPI system in rodent cerebellum appears to be driven by activation of a QA receptor[5]. Consequently, our finding of diminished QA receptors in OPCA molecular layer and their probable location on Purkinje cell dendrites supports the inference of Kish et al. that the human cerebellar PPI system is localized in Purkinje cell dendrites.

Our data from OPCA specimens also have implications for efforts to image cellular changes in human neurodegenerative diseases in vivo with positron emis-

sion tomography (PET). The marked reductions we found in cerebellar cortical neurotransmitter receptors suggest that PET ligands for neurotransmitter receptors may be able to detect cellular changes in OPCA patients in vivo. BDZ receptors, by virtue of their relatively high density and the availability of high affinity ligands that cross the blood brain barrier (BBB), may be good targets for initial studies. In preliminary experiments, we have demonstrated depletion of BDZ receptors in OPCA with the BDZ ligand Ro15-1788 (Albin et al., unpublished data), a compound already used successfully in humans for PET visualization of BDZ receptors[40]. Because of their overall high density in cerebellar cortex, GABA$_A$ and QA receptors may also be good targets if high affinity ligands that cross the BBB can be developed.

In summary, we have studied the distribution of BDZ receptors, GABA$_A$ receptors, GABA$_B$ receptors, QA receptors, NMDA receptors, PCP receptors, and non-NMDA/non-QA [^{3}H]GLU binding sites in normal human and OPCA cerebellar cortex. BDZ, GABA$_A$, and NMDA receptors have a relatively high density in the granule cell layer while BDZ, GABA$_B$, QA and the non-NMDA/non-QA [^{3}H]GLU binding sites have a relatively high density in the molecular layer. Our study of OPCA specimens is consistent with the localization of QA, GABA$_A$, and BDZ receptors on Purkinje cell dendrites and NMDA and GABA$_A$ receptors on granule cells in human cerebellar cortex. GABA$_B$ receptors may be on Purkinje cell dendrites, parallel fiber terminals, or climbing fibers. Our findings are concordant with prior experimental animal data and indicate that the synaptic organization of human cerebellar cortex is largely similar to that of other mammals, although the presence of relatively high densities of BDZ receptors in the granule cell layer and the non-NMDA/non-QA [^{3}H]GLU binding site in the molecular layer indicate some features different from rodents.

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