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AUTORADIOGRAPHIC LOCALIZATION OF CEREBELLAR EXCITATORY AMINO ACID BINDING SITES IN THE MOUSE

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Abstract—We have investigated the cellular localization of cerebellar excitatory amino acid binding sites in normal mice, in mice deficient in granule cells and, perhaps, stellate, basket and Golgi cells (granuloprival mice) and in mice lacking Purkinje cells. In the molecular layer of normal mouse cerebellum, the quisqualate-sensitive binding sites were the predominant type of excitatory amino acid receptor and there were relatively few *N*-methyl-D-aspartate or kainate-sensitive binding sites. The granule cell layer of normal mice contained a mixture of all 3 types, the *N*-methyl-D-aspartate-sensitive binding sites being predominant.

In the molecular layer of granuloprival mice, the number of quisqualate-sensitive binding sites was increased to 214% of control ($P < 0.01$), whereas *N*-methyl-D-aspartate-sensitive binding sites were decreased to 62% of control ($P < 0.001$) and kainate-sensitive binding sites were unchanged. In the granule cell layer of these mice, quisqualate-sensitive binding sites were increased to 200% ($P < 0.01$), *N*-methyl-D-aspartate-sensitive binding sites were decreased to 47% ($P < 0.001$) and kainate-sensitive binding sites were decreased to 49% ($P < 0.01$) of their respective control values.

In the molecular layer of mice lacking Purkinje cells, quisqualate-sensitive binding sites were reduced to 29% ($P < 0.001$) of control and *N*-methyl-D-aspartate-sensitive binding sites were unchanged. In the granule cell layer of these mice, neither quisqualate nor *N*-methyl-D-aspartate-sensitive binding sites were changed.

These results suggest that (1) quisqualate-sensitive binding sites are located principally on dendrites of Purkinje cells and that they up-regulate after deafferentation; (2) *N*-methyl-D-aspartate-sensitive binding sites are located on granule cells and, perhaps, stellate, basket and Golgi cells, and (3) kainate binding sites are located on cell bodies of granule and, perhaps, Golgi cells.

Glutamate, aspartate and perhaps some closely related compounds are thought to be major excitatory neurotransmitters in the central nervous system.[15,52] The actions of these excitatory amino acids are mediated by at least three distinct receptors named for the selective agonists *N*-methyl-D-aspartate (NMDA), quisqualate (QA) and kainate (KA).[36,51,52] The existence of multiple classes of binding sites, all of which bind glutamate, raises the possibility that each class of binding sites may have a distinct function and/or that each has a different neuronal or regional localization. Recently, autoradiographic methods have been developed to localize, characterize and quantify these binding sites.[22,24,25,39]

The cerebellar cortex is a convenient region in which to study excitatory amino acid binding sites because of its relatively simple, laminar cytoarchitecture, anatomically and physiologically defined circuitry and neurochemically defined cell types. In addition, there are selective mutations and cytotoxic drug treatments which render the cerebellum deficient in specific cell types, thereby altering cerebellar circuitry. Most importantly, it has been proposed that excitatory amino acids are the transmitters of the afferent climbing fibers,[17,44,54] mossy fibers[4,18] and the intrinsic granule cell/parallel fiber system.[28,45,56] Thus, the dendrites of the Purkinje cells receive excitatory amino acid input from both the climbing fibers and the granule cell/parallel fiber system whereas the granule cells receive such input from the mossy fibers. In this study, using normal mice, granuloprival mice and mice lacking Purkinje cells, we have endeavored to determine the cellular localization of cerebellar NMDA-, QA- and KA-sensitive binding sites by means of quantitative receptor autoradiography.

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Abbreviations: AP4,2-amino-4-phosphonobutyrate; GRN, granule cell layer; KA, kainate; MAM, methylazoxymethanol acetate; NMDA, *N*-methyl-D-aspartate; QA, quisqualate.

EXPERIMENTAL PROCEDURES

Animals

Granuloprival mice were produced by injecting pups of BLU-HA mice (Blue Spruce Farms, Albany, NY) with

methylazoxymethanol (MAM, 30 mg/kg diluted to a concentration of 10 mg/ml in 0.9% NaCl) subcutaneously within hours of birth. MAM is a nucleic acid alkylating agent which kills cells undergoing mitosis at the time of drug distribution.[30] When injected on postnatal day 1, this agent causes a selective depletion of granule, stellate, basket and Golgi cells in the mouse cerebellum. Binding experiments were performed in 42-day-old mice that had been treated as pups with MAM. Noninjected littermates were used as controls. Homozygous, recessive "nervous" mutant mice (nr/nr) which lack Purkinje cells[32] were obtained from Jackson Laboratories (Bar Harbor, ME). Their neurologically normal littermates served as controls.

Tissue preparation

Mice were decapitated and their brains were rapidly removed. Cerebella and brainstems were dissected from forebrain, mounted on cryotome chucks with Lipshaw embedding matrix, and frozen under powdered dry ice. The mounted cerebella were warmed to −20°C and 20 μm sections were cut and thaw-mounted onto gelatin-coated slides. Tissue sections were washed in either 50 mM Tris–HCl containing 2.5 mM $CaCl_2$ or 50 mM Tris–acetate (pH 7.2 at 2°C) for 30 min at 2°C to remove endogenous competitors and then were dried under a stream of room temperature air. The buffer used in this prewash (Tris–HCl or Tris–acetate) was always the same as that used in subsequent binding experiments and in the final rinse, as described below.

Autoradiography

A detailed description of the method for glutamate receptor autoradiography has been published.[22,24,55] Briefly, in glutamate saturation studies, tissue sections were incubated with 30 nM L-[^{3}H]glutamate (sp. act. 39 Ci/mmol) and varying concentrations of unlabeled glutamate ranging from 1 nM to 10 μM Tris–HCl containing 2.5 mM $CaCl_2$ for 45 min at 2°C. Nonspecific binding was determined in the presence of 1 mM unlabeled glutamate and represented <10% of total binding. Similar "blanks" were obtained with 1 mM QA. Detailed competition studies were carried out in the presence of 200 nM glutamate (sp. act. 4.5 Ci/mmol) using a wide range of QA concentrations 1 nM–1 mM) or NMDA concentrations (100 nM–1 mM). Previous studies have shown that QA displaces all specific glutamate binding biphasically with K_is for the high affinity and low affinity sites that differ by more than 10,000-fold.[22,24] The site with a low affinity for QA is equivalent to the NMDA site.[22] QA binding sites are defined, therefore, by their high affinity for QA in detailed competition curves using the LIGAND program to resolve the sites as described previously.[22,24,40] Similar results were obtained by defining QA sites as the specific glutamate binding sites remaining in the presence of 100 μM NMDA or those specific binding sites displaced by 2.5 μM QA. In analogous fashion, NMDA binding sites were defined as either those glutamate binding sites having a low affinity for QA in detailed competition curves or those sites remaining in the presence of 2.5 μM QA. The glutamate binding assay did not measure kainate-sensitive sites because binding to these sites is inhibited by the calcium present in the assay buffer.[22,39] Kainate binding studies were carried out at 2°C with 65 nM [^{3}H]kainate (sp. act. 5 Ci/mmol) in 50 mM Tris–acetate (pH 7.2) with various concentrations of unlabeled kainate (1 nM–100 μM) for saturation studies. Nonspecific binding was determined in adjacent sections in the presence of either 1 mM unlabeled glutamate or 100 μM unlabeled kainate and represented <5% of total binding.

After the incubation, sections were quickly rinsed 3 times with cold buffer, then rinsed once with cold 2.5% glutaraldehyde in acetone. The total rinse time was <10 s. Slides were blown dry with warm air, placed in X-ray cassettes with appropriate radioactive standards and apposed to LKB Ultrofilm ^{3}H.[42] The film was exposed to the tissue sections for 14–21 days at 4°C, then developed, fixed and dried. Tissue sections were then post-fixed with paraformaldehyde and stained with Cresyl Violet. Individual layers of cerebellum were identified on autoradiographs by comparison with the Cresyl Violet stained sections of the tissue that produced the film image. The optical densities of the film images were determined using a computer-assisted microdensitometer.[10] Twenty or more readings from each layer of interest were averaged and the radioactivity of the underlying tissue determined by a computer-generated polynomial regression analysis which compared film densities produced by the tissue sections to those produced by the radioactive standards.[42] All binding data were obtained by microdensitometry.

Data analysis

Binding to the molecular and granule layers of MAM-treated mice was compared to that in untreated littermates. Similarly, binding in homozygous "nervous" mutant mice lacking Purkinje cells was compared to neurologically normal littermates. Thus, MAM-treated and "nervous" mutant mice were compared to separate sets of control animals. Statistical analysis was by unpaired *t*-tests.

RESULTS

Histology

In normal cerebellum the granule cell layer is characterized by a very dense collection of cell nuclei. The Purkinje cell layer is a monolayer of large neurons with pale nuclei located between the granule cell layer (GRN) and the molecular layer. The molecular layer has relatively few intrinsic neurons (basket and stellate cells) and the abundant neuropil consists largely of granule cell axons and Purkinje cell den-

Fig. 1. Nissl-stained sections of control cerebellum (A), cerebellum rendered deficient in granule cells by MAM treatment (B), and cerebellum of "nervous" mutants lacking Purkinje cells (C). In control cerebellum the GRN is characterized by densely packed granule cell bodies with prominent nuclei. The Purkinje cells are arranged in a monolayer between the GRN and the molecular layer. The molecular layer has relatively few intrinsic neurons and abundant neuropil consisting largely of granule cell axons and Purkinje cell dendrites. In MAM-treated mice there was marked depletion of granule cells in a patchy distribution most prominent in midline regions. In affected areas, the GRN appeared wider than normal but with a decreased density of granule cell bodies (black arrows). The molecular layer was slightly narrower than in controls but there was no glial proliferation; Purkinje cells appeared normal. Glutamate binding data in these mice were obtained from the midline regions of severe granule cell depletion and from adjacent areas of the molecular layer (white arrows). In "nervous" mutant mice there were very few Purkinje cells. The GRN was normal and the molecular layer was slightly thinner than normal, with an increase in the relative density of Bergman astrocytes. MOL, molecular layer.

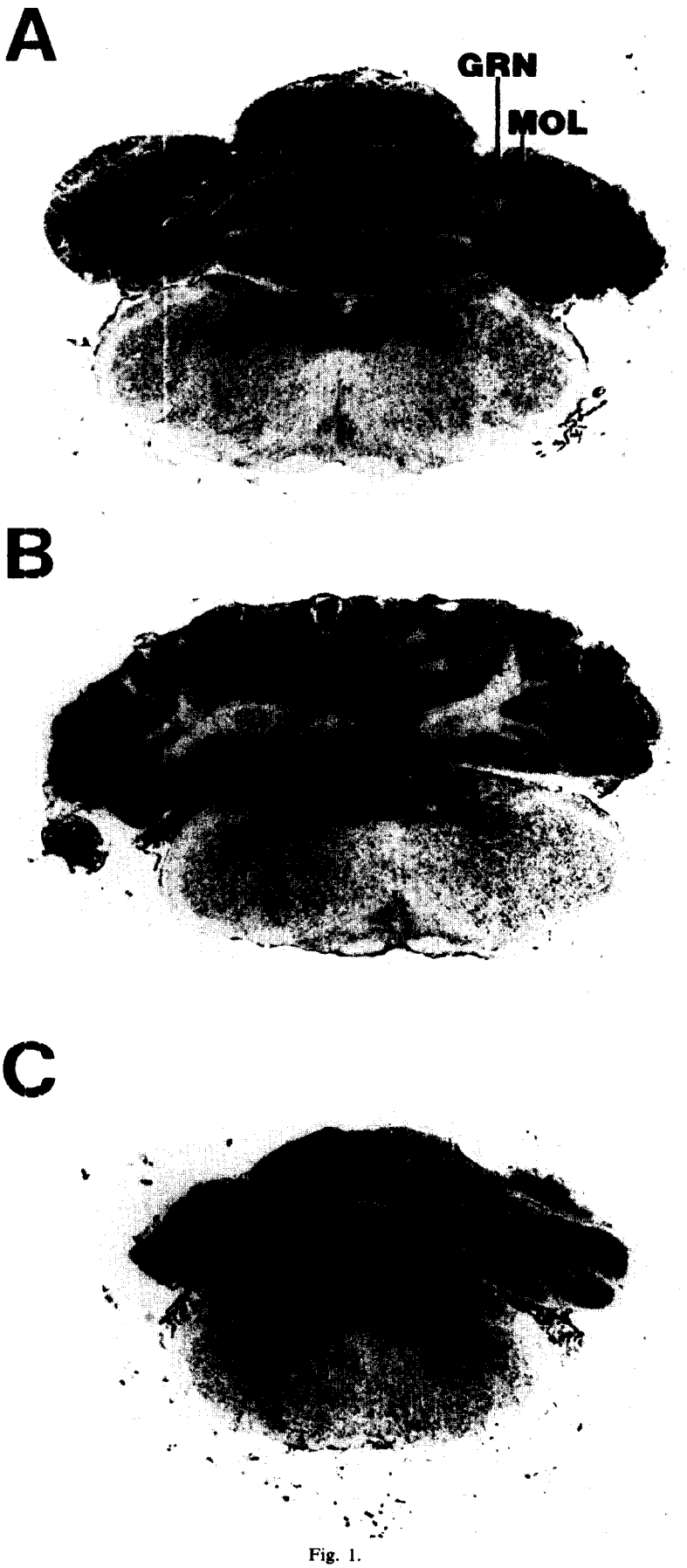

Fig. 1.

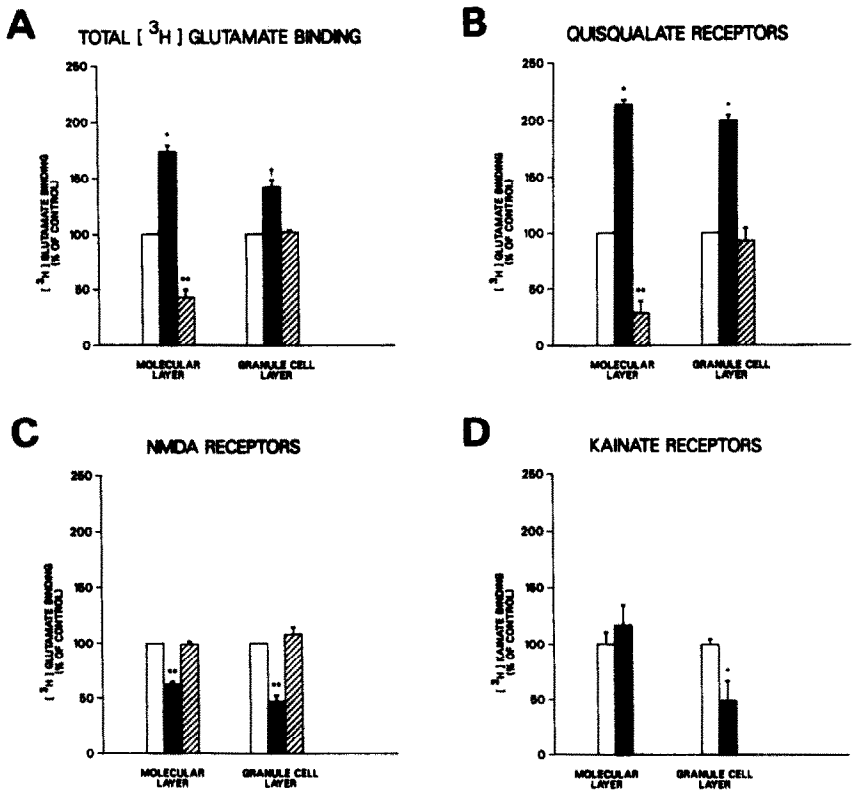

Fig. 3. Histograms of total glutamate binding (A), QA receptors (B), NMDA receptors (C) and KA binding (D) in the molecular layer and the GRN of granule cell-deficient mice (solid bars) and mice lacking Purkinje cells (striped bars) compared to controls (open bars). Data is presented as percentage of control values ± S.E.M. In experiments represented by histograms (A), (B) and (C), separate sets of control animals were used for comparison with the MAM-treated and "nervous" mutants, as described under Experimental Procedures. Thus, control bars do not show standard error because they represent the level of binding (100%) in two separate sets of control animals. In (D), control bars do show standard error because only data from MAM-treated mice and their controls are shown. Levels of significance ($\dagger P < 0.05$, $*P < 0.01$, $**P < 0.001$) were determined by comparison with appropriate controls by unpaired t-tests as described under Experimental Procedures. [^{3}H]Glutamate concentration was 200 nM (A–C) and [^{3}H]kainate concentration was 65 nM (D).

drites (Fig. 1A). In the MAM-treated rats, there was a marked depletion of granule cells in a patchy distribution (Fig. 1B). The areas of granule cell depletion were most prominent in the midline regions and the damaged granule cell layer appeared wider than normal, but had a markedly decreased density of granule cell nuclei. The molecular layer was slightly narrower than in normal rats but had no increase in the number of glial cell nuclei. Purkinje cells in these animals appeared normal. In "nervous" mutant mice (nr/nr), there was a severe depletion of Purkinje cells. The GRN was normal, whereas the molecular layer was somewhat thinner than normal (Fig. 1C) with an increase in the relative density of Bergman astrocytes.

Total glutamate binding

At a [^{3}H]glutamate concentration of 200 nM, total glutamate binding in control mice was higher in the molecular layer than in the GRN (5.55 vs 4.19 pmol/mg protein, respectively) (Figs 2 and 3). In MAM-treated mice, regions of severe granule cell depletion had increased levels of binding. At 200 nM, glutamate binding was increased to 174% of control in the molecular layer and to 140% in the GRN ($P < 0.01$ and $P < 0.05$, respectively). Saturation analysis in the molecular layer of control and MAM-treated mice (Fig. 4) revealed no significant difference in affinity of binding (K_Ds: 1.5 ± 0.1 vs 1.5 ± 0.4 μM, respectively) but a large increase in the number of

Fig. 2. Autoradiographs of total L-[^{3}H]glutamate binding (left), QA receptors (center) and NMDA receptors (right) in control cerebellum (top row), granule cell-deficient cerebellum (middle row) and Purkinje cell-deficient cerebellum (bottom row). Black arrows mark areas of the GRN and white arrows mark areas of the molecular layer from which binding data were obtained. Receptor binding data are presented under Results and in Figs 3–5. MOL, molecular layer.

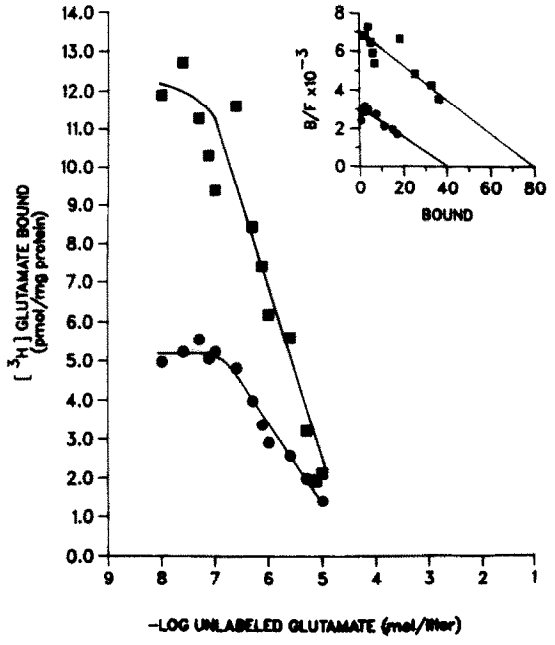

Fig. 4. Saturation analysis of L-[^{3}H]glutamate binding in the molecular layer of control (circles) vs granule cell-deficient mice (squares). Inset shows Scatchard plots of the data. Saturation analysis was performed as described under Experimental Procedures. Glutamate binding in control and granule cell-deficient mice has the same affinity, but there was a large increase in the number of binding sites (B_{max}, in pmol/mg protein) in the MAM-treated animals. See text for details.

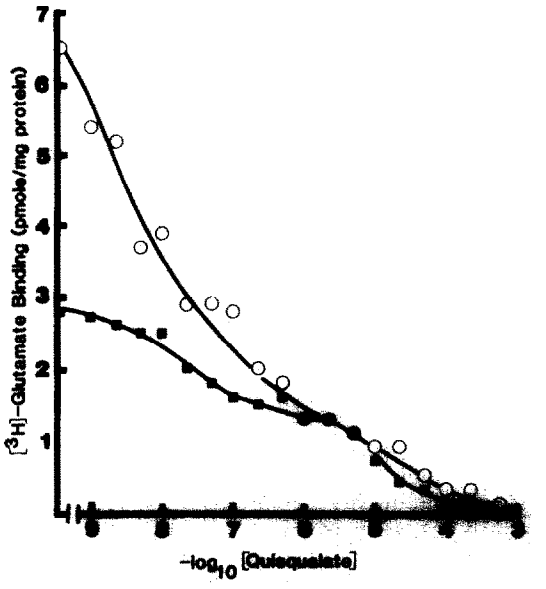

Fig. 5. Quisqualate competition curves in the molecular layer of control mice (circles) and "nervous" mutant mice which lack Purkinje cells (squares). Analysis of QA competiton data reveals 2 sites with different affinities for QA. In the "nervous" mutants, there is a profound loss of glutamate binding sites that have a high affinity for QA (see text and Table 1).

binding sites in MAM-treated mice (B_{max}: 43 ± 3 vs 91 ± 20 pmol/mg protein, respectively $P < 0.01$). In contrast, in the "nervous" mutant mice which lack Purkinje cells total glutamate binding was decreased to 43% of control in the molecular layer ($P < 0.001$) and was unchanged in the GRN.

Quisqualate-sensitive binding sites

In control mice, QA-sensitive binding sites (measured in the presence of 100 μM NMDA) were almost three times as numerous in the molecular layer as they were in the GRN (3.98 vs 1.40 pmol/mg protein, respectively) (Figs 2 and 3). Under the conditions of the binding assay, QA-sensitive binding sites account for about 75% of specific glutamate binding sites in the molecular layer of control mice (Fig. 5 and Table 1). Only about 30% of GRN binding sites are QA-sensitive. In the molecular layer of MAM-treated mice, QA-sensitive sites were increased to 214% of control ($P < 0.01$) in regions adjacent to severe granule cell depletion (Figs 2 and 3). QA-sensitive sites were increased to 200% of control in the GRN of these mice ($P < 0.01$). In the mice lacking Purkinje cells ("nervous" mutants), there was a marked loss of QA binding sites in the molecular layer, where the Purkinje cell dendrites are located (Figs 2 and 3). QA-sensitive binding sites measured as glutamate binding in the presence of 100 μM NMDA, showed a reduction to 29% of control in the molecular layer ($P < 0.001$); QA sites were unchanged in the GRN of these mice. Similarly, detailed QA competition curves, analysed by an iterative curve-fitting computer program,[40] showed that QA binding sites were decreased to 24% of control ($P < 0.005$) in the molecular layer of "nervous" mutants (Fig. 5 and Table 1).

N-Methyl-D-aspartate-sensitive binding sites

There were more NMDA-sensitive binding sites in

Table 1. Comparison of quisqualate binding parameters in control mice and mice lacking Purkinje cells

	K_H (nM)	K_L (μM)	R_H (pmol/mg protein)	R_L
Control	114 ± 60	175 ± 64	25 ± 1	7 ± 1
Nervous	25 ± 6	53 ± 45	6 ± 3*	7 ± 2

Data represent the mean ± S.E.M. of 3 separate experiments (3 control and 3 nervous mice) performed in Tris-HCl containing 2.5 mM $CaCl_2$. Data were analysed by the LIGAND program.[40] Two site fits were significantly better than one site fits in all cases. *$P < 0.005$ by unpaired t-test.

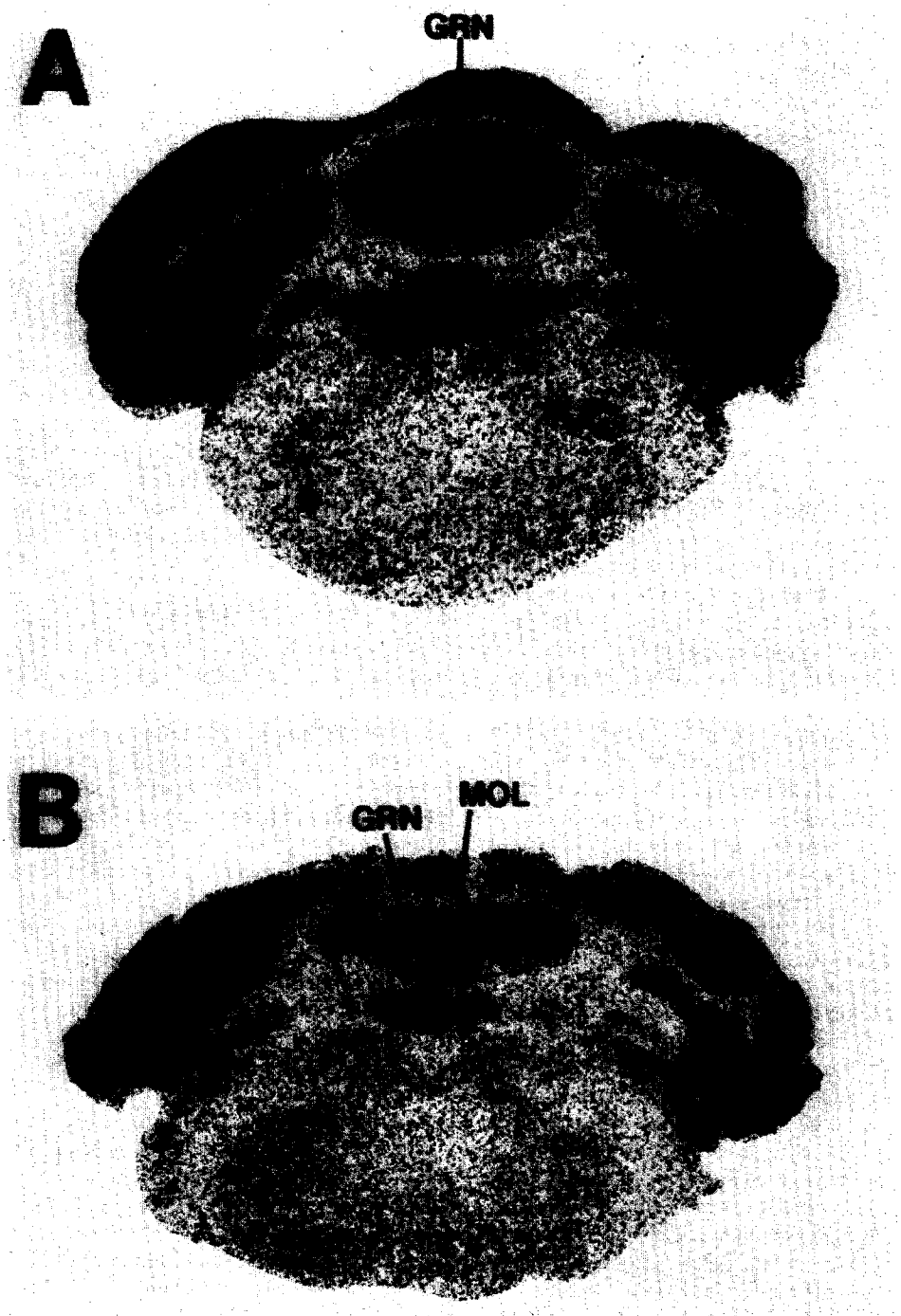

Fig. 6. Autoradiographs of kainate binding in control cerebellum (A) and granule cell-deficient cerebellum (B). Control mice have more KA binding in the GRN than in the molecular layer. In mice lacking granule cells, KA binding is reduced in the GRN and unchanged in the molecular layer (see text and Fig. 3D). MOL, molecular layer.

the GRN than in the molecular layer of control mice (3.57 vs 1.58 pmol/mg protein, respectively) (Figs 2 and 3). In the GRN of MAM-treated mice, NMDA-sensitive binding sites were decreased to 47% of control ($P < 0.001$) (Figs 2 and 3). NMDA sites were also decreased to 62% of control ($P < 0.001$) in the molecular layer of MAM-treated mice (Figs 2 and 3). Mice lacking Purkinje cells had normal levels of NMDA binding sites in both GRN and the molecular layer (Figs 2 and 3).

Kainate receptors

KA binding sites, assayed with [^{3}H]kainate, were more dense in GRN than in the molecular layer in normal mouse cerebellum (1.22 vs 0.65 pmol/mg protein, respectively) (Fig. 6). MAM treatment did not affect KA binding in the molecular layer but decreased the number of sites to 49% of control in the GRN ($P < 0.01$). KA binding sites were not assayed in the "nervous" mutants lacking Purkinje cells.

DISCUSSION

The cerebellar cortex is a relatively uniform, laminar structure contain five intrinsic neuronal types—granule, Purkinje, Golgi, stellate and basket cells—which are arranged in three distinct layers.[29,41] The deepest layer, the GRN, consists of numerous, densely packed granule cell bodies and dendrites and less common Golgi cell bodies. The middle layer, situated just outside the GRN, is composed of a single layer of Purkinje cell bodies. The dendritic arbor of the Purkinje cells is located in the most superficial layer, the molecular layer. Axons of the granule cells project from the GRN into the molecular layer and there, divide to form the parallel fibers which make extensive synaptic contacts with the Purkinje cell dendrites. The stellate and basket cells and Golgi cell dendrites are also located in the molecular layer and, to a lesser extent, also receive synaptic input from the parallel fibers. The granule cells are the only intrinsic cerebellar cells which are excitatory,[29,41] and extensive evidence suggests that glutamate is the transmitter of the granule cell/parallel fiber system.[28,45,56]

Afferents to the cerebellar cortex, the mossy fibers and the climbing fibers, are excitatory.[11] Recent evidence suggests that the mossy fibers[4,18] and the climbing fibers[17,44,54] may also use excitatory amino acid neurotransmitters. Mossy fibers, from brainstem and spinal cord, synapse in the GRN on the dendrites of granule cells. Climbing fibers synapse in the molecular layer on Purkinje cells. Thus, the dendrites of Purkinje cells receive excitatory amino acid input from both the granule cell/parallel fiber system and the climbing fibers while stellate, basket and Golgi cells receive input from the granule cell parallel fiber system only. Similarly, the granule cells receive such input from the mossy fibers. It might be expected, then, that all cerebellar cortical neurons would have binding sites for excitatory amino acids.

The cellular location of excitatory amino acid receptors in mouse cerebellum can be determined by using mice which lack selected cell types. When mice are treated within 24 h of birth with MAM, the chemical kills dividing granule cells by alkylating nucleic acids.[30] Stellate, basket and Golgi cells may be involved to a lesser extent.[1-3,26,35,48] The resulting cerebellum is hypoplastic, with a marked loss of granule cells and a molecular layer containing disorganized Purkinje cells. There is no apparent increase in glial cell number and only slight swelling of their processes despite massive neuronal loss. Except for abnormal orientation, and the possible presence of some aberrant Purkinje cell dendrites in the GRN, Purkinje cells appear entirely normal, with intact postsynaptic dendritic spines.[27,31,33,46] The dendritic spines of Purkinje cells are the exclusive sites of parallel fiber synapses onto these cells.[29,30] The net result of this treatment is a relatively selective destruction of glutamatergic granule cells and deafferentation of Purkinje cells which retain normal dendritic spines in the molecular layer. In contrast, "nervous" (nr/nr) is an autosomal recessive mutation of BALB/cGr inbred mice which results in selective degeneration of approximately 90% of Purkinje cells.[47] The use of MAM-treated and "nervous" mutant mice allows determination of the number and type(s) of excitatory amino acid receptors located on non-Purkinje and Purkinje cells, respectively.

Quisqualate-sensitive binding sites

Approximately 75% of glutamate binding in the molecular layer of normal mice is associated with binding to QA-sensitive sites, as reported previously.[22,24] Granule cell/parallel fiber synapses on Purkinje cell dendrites are the most numerous synapse in the molecular layer.[27] It is therefore possible that the most abundant type of binding site in the molecular layer, the QA binding site, is associated with the type of excitatory amino acid synapse most numerous in this region. In support of this hypothesis that QA binding sites mediate the parallel fiber–Purkinje cell synapse, QA-sensitive binding site data (Fig. 3A) indicate decreased QA-sensitive binding in the absence of Purkinje cells. Because the density of Bergman astrocytes increases in the molecular layer of nervous mice, the observed decrease in binding to this layer could be due to greater absorption of beta emission by glia.[21] This possibility is unlikely though since there is no change in NMDA binding site density in the molecular layer of these same brains. Thus, QA binding sites appear to be located on Purkinje cells. The remaining QA binding sites in the molecular layer are probably located on the 10% of Purkinje cells unaffected by this mutation and on remaining basket, stellate and Golgi cells with which the parallel fibers also synapse.

We have also found that when Purkinje cells are deafferented by granule cell ablation in MAM-treated mice, there is an apparent up-regulation of QA binding sites in areas of the molecular layer adjacent to the regions of greatest granule cell ablation. This is consistent with the preservation of normal postsynaptic dendritic spines on the Purkinje cells of MAM-treated mice.[27] Although we cannot rule out entirely the possibility that this up-regulation merely reflects a relative concentration of Purkinje cell dendrites due to parallel fiber loss, this appears unlikely

since NMDA binding sites are decreased and kainate binding sites are unchanged by this treatment (see below). It is noteworthy that Slevin *et al.*[48] reported a 65% increase in glutamate binding in mouse cerebellar homogenates following MAM treatment. In addition, physiological evidence for denervation supersensitivity at a central glutamatergic synapse has recently been described.[49] Binding to QA-sensitive sites in the molecular layer adjacent to areas of the GRN which were not affected by MAM treatment was unchanged from controls (data not shown). This suggests that the increase in binding sites is due to granule cell loss rather than MAM treatment itself. Although the 40 μm resolution of this receptor autoradiographic method is insufficient to provide a definite answer, the data from "nervous" and MAM-treated mice suggests that QA binding sites are associated with the parallel fiber–Purkinje cell synapse. The results of physiological and biochemical studies which attempt to mimic the actions of the parallel fiber–Purkinje cell synapse *in vivo* are consistent with this interpretation.[8,9,16]

QA-sensitive binding sites are also found in the granule cell layer of cerebellar cortex. However, it is unlikely that these sites are associated with granule cells, because granule cell ablation actually leads to an increase in QA sites in the GRN. Most likely this is due to "the presence of aberrant Purkinje cell dendrites among the granule cells" postulated by Lovell and coworkers.[33] In "nervous" mutant mice QA-sensitive sites were not changed in the GRN. Binding to the QA site is dependent on both calcium and chloride ions.[22] Recently, a chloride-dependent, quisqualate-sensitive glutamate binding site, possibly a transport site, has been described in astrocyte membranes.[5] It is unlikely that this site is measured in our assay because binding to the astrocytic site is highly temperature-dependent and no specific binding can be measured at 2°C, the temperature at which our assay is performed. Also, the QA binding site described in the present study decreases in other regions of glial proliferation such as kainate-lesioned striatum (unpublished observations) or striatum of patients dying with Huntington's disease.[23] Finally, the affinity of quisqualate for the QA binding site described here is at least 1000-fold higher than that of the astrocytic site.[5]

It should also be noted that the QA binding site described in this report is not the 2-amino-4-phosphonobutyrate (AP4)-sensitive glutamate binding site described in fresh homogenates.[12,53] The AP4-sensitive site, which is chloride-dependent and calcium-enhanced, is destroyed by freezing,[13,53] and is therefore not present in autoradiographic assays.[22,39] Furthermore, AP4 has no effect on glutamate binding in our assay.[22]

In summary, the evidence suggests that the QA-sensitive binding site described here is located on neurons, can up-regulate in response to deafferentation, and corresponds to the dendritic QA receptor defined electrophysiologically in the molecular layer of the cerebellum.

N-*Methyl*-D-*aspartate-sensitive binding sites*

In cerebellar cortex, NMDA-sensitive binding sites are more abundant in the GRN than in the molecular layer, as reported previously.[22,24,37] The bulk of NMDA binding sites in the GRN appear to be associated with granule cell dendrites because MAM treatment results in a 53% reduction of NMDA sites. These binding sites may be postsynaptic to mossy fibers which, based on immunocytochemical data, may be glutamatergic.[4] Alternatively, if Golgi neurons are destroyed by MAM treatment then the NMDA sites may be located on Golgi neurons.

In animals treated with MAM there is a reduction in NMDA binding sites in the molecular layer (Fig. 3B). Destruction of stellate and, perhaps, basket cells by MAM in this layer suggests that NMDA binding sites are located on stellate and basket cells. As with NMDA sites in the GRN, the NMDA sites in the molecular layer might be located on Golgi neurons if these cells are destroyed by MAM. Localization of NMDA binding sites to stellate cells and possibly to basket and Golgi cells is in agreement with electrophysiological studies in which NMDA has prominent effects on inhibitory interneurons when applied iontophoretically.[9,43] Furthermore, the only cells in the cerebellar cortex which are vulnerable to NMDA toxicity are stellate, Golgi, and basket cells.[19]

Another possibility is that a portion of the NMDA sites are located on axon terminals (parallel fibers) of granule cells. In MAM-treated cerebella, there is no change in NMDA binding in the molecular layer adjacent to the GRN in which granule cells were unaffected by MAM. The data demonstrating decreased NMDA-sensitive binding (Fig. 3B) was obtained by densitometrically analysing the molecular layer adjacent to substantial granule cell ablation. Presynaptic NMDA receptors have been postulated in olfactory cortex based on glutamate release studies[7] and in hippocampus based on electrophysiological studies[6] and glutamate release.[34] The present study contains the first binding data suggestive of presynaptic NMDA receptors.

In "nervous" mutant mice lacking Purkinje cells, NMDA binding sites were unchanged in the molecular layer. This provides strong evidence that few, if any, NMDA binding sites are located on the dendrites of Purkinje cells and further strengthens the supposition that QA receptors mediate synaptic transmission of the parallel fibers. Electrophysiological data also suggest that there are few NMDA receptors on Purkinje cells. Crepel *et al.*[9] recorded from Purkinje cells during iontophoretic dendritic application of excitatory amino acids and found that NMDA was very weak or inactive in this region, whereas QA was a very potent excitant.

Kainate-sensitive binding sites

The distribution of KA binding sites in this study is similar to that reported by others,[38,50] with higher levels of binding found in the GRN than in the molecular layer. KA binding sites were reduced by half in the GRN of MAM-treated mice, indicating that these binding sites are likely associated with granule cell dendrites. KA binding in the molecular layer was unaffected by granule cell ablation. This suggests that there are not presynaptic KA binding sites on parallel fibers and argues against a role for such KA receptors at granule cell axon terminals in controlling glutamate release.[14] Future studies, using mice lacking climbing fibers or mossy fibers, may elucidate the site of action of kainate in mediating excitatory amino acid release. The question of whether there are KA binding sites located on Purkinje cells was not addressed in the present study, although there is physiological evidence to this affect.[20]

CONCLUSIONS

In this study we have used quantitative autoradiography to localize excitatory amino acid binding sites in the cerebellum. We have strengthened the contention that QA binding sites mediate the responses of the parallel fiber–Purkinje cell synapse. Our data further suggest that these binding sites up-regulate in response to deafferentation. We have also provided evidence that a population of NMDA binding sites is located on granule and stellate cells and, perhaps, on basket and Golgi cells. Granule cells also appear to have KA binding sites. This information should help to better define the roles and sites of action of excitatory amino acids in the cerebellum.

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