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Excitatory and inhibitory amino acid binding sites in human dentate nucleus

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Autoradiography of excitatory and inhibitory amino acid binding sites in human dentate nuclei indicated virtually no binding to *N*-methyl-D-aspartate (NMDA) or γ-aminobutyric acid$_B$ ($GABA_B$) binding sites, and a low density of kainate binding sites. α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid, metabotropic-quisqualate, benzodiazepine, and γ-aminobutyric acid$_A$ ($GABA_A$) binding sites were present in moderate abundance. Our NMDA results differ from those found previously in rodents. $GABA_A$ receptors are probably the primary mediators of inhibitory neurotransmission and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and metabotropic-quisqualate receptors are probably the primary mediators of excitatory neurotransmission within the human deep cerebellar nuclei.

The deep cerebellar nuclei (DCN) give rise to virtually the only projection conveying information out of the cerebellum. The inhibitory amino acid γ-aminobutyric acid (GABA) and the excitatory amino acids (EAAs) are important neurotransmitters within the DCN. The DCN receive GABAergic afferents from the Purkinje cells of the cerebellar cortex and EAAergic input from collaterals of mossy and climbing fibers innervating the cerebellar cortex[4,7,10,12,21,23,24,27]. In addition, the DCN contain local interneurons that are thought to be GABAergic[7,19]. DCN neurons projecting out of the cerebellum are thought to be EAAergic[18] and have recurrent collaterals within the DCN[7].

An important aspect of both GABA- and EAA-mediated neurotransmission is the existence of receptor subtypes with distinctive physiological properties. Two types of GABA receptors are recognized. The $GABA_A$ receptor is an ionotropic receptor coupled to an inhibitory chloride channel and modulated by an integral benzodiazepine (BDZ) binding site[9]. The $GABA_B$ receptor is a G-protein-coupled receptor that modulates potassium and calcium channels[5]. Four types of well defined EAA receptors are presently recognized[15]. The *N*-methyl-D-aspartate (NMDA) receptor is an ionotropic receptor that produces long latency, long duration depolarizations and is linked to an ionophore that permits calcium influx. The NMDA receptor complex is also distinguished by the presence of additional binding sites for glycine (Gly), the dissociative anesthetics, and polyamines. The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor (known also as the ionotropic-quisqualate receptor) and the kainate (KA) receptor mediate conventional fast neurotransmission. The metabotropic-quisqualate (MET) receptor is linked to the inositol phospholipid second messenger system. To explore the role of GABA and EAAs within the human DCN, we used receptor autoradiography to determine which GABA and EAA binding site subtypes were present within the dentate nucleus (DN) of man.

Six brains were obtained at necropsy from individuals without neurologic disease. The average age was 56 years (range 31–69) and the average post-mortem delay was 13 h (range 10–16). Brains were bisected in the sagittal plane; one hemisphere sectioned coronally into 1 cm slabs; the slabs frozen in crushed dry ice and stored at −70 °C. Blocks containing the dentate nucleus (DN) and cerebellar cortex were cut out of the slabs and 20 micron sections were cut with a Lipshaw cryostat. Sections were thaw mounted onto gelatin coated slides and stored at −20 °C for 1 to 2 days.

EAA and GABA binding site subtypes were assayed with standard techniques (Table I)[1,6,8,13,20,22,25,28]. All assays were run in triplicate or duplicate. $GABA_A$, $GABA_B$, NMDA, AMPA, MET, KA, and GLY binding sites were all assayed in a similar manner. Sections were prewashed in buffer at 4 °C, dried under a stream

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TABLE I

GABA and EAA binding site assays

Binding site	*Ligand*	*Specific activity*	*Conc.*	*Buffer*	*Blockers*	*Blank*
BDZ	[^{3}H]Flunitrazepam	85 Ci/mmol	5 nM	50 mM Tris-acetate pH 7.4, 4 °C		5 μM Clonazepam
$GABA_A$	[^{3}H]GABA	52 Ci/mmol	20 nM	50 mM Tris-Cl + 2.5 mM $CaCl_2$ pH 7.2, 4 °C	100 μM Baclofen	100 μM Isoguvacine
$GABA_B$	[^{3}H]GABA	52 Ci/mmol	20 nM	50 mM Tris-Cl + 2.5 mM $CaCl_2$ pH 7.4, 4 °C	10 μM Isoguvacine	100 μM Baclofen
NMDA	[^{3}H]Glutamate	56 Ci/mmol	65 nM	50 mM Tris-acetate pH 7.4, 4 °C	2.5 μM Quisqualate + 1 μM Kainate	1 mM NMDA or 100 μM CPP
Glycine	[^{3}H]Glycine	18.7 Ci/mmol	100 nM	50 mM Tris-acetate pH 7.4, 4 °C	1 μM Strychnine	1 mM Glycine
MK-801	[^{3}H]MK-801	22.5 Ci/mmol	10 nM	50 mM Tris-acetate pH 7.4, 25 °C + 30 μM Glycine + 100 μM Glutamate		10 μM MK-801
AMPA	[^{3}H]AMPA	29.2 Ci/mmol	35 nM	50 mM Tris-Cl + 2.5 mM $CaCl_2$ + 30 mM KSCN pH 7.2, 4 °C		1 μM Glutamate
MET	[^{3}H]Glutamate	56 Ci/mmol	100 nM	50 mM Tris-Cl + 2.5 mM $CaCl_2$ + 30 mM KSCN pH 7.2, 4 °C	100 μM NMDA + 10 μM AMPA	2.5 μM Quisqualate
Kainate	[^{3}H]Kainate	5.9 Ci/mmol	60 nM	50 mM Tris-acetate pH 7.2, 4 °C		100 μM Kainate

of cool air and immersed in cytomailers containing buffer (4 °C) with radioactive ligand and selective blocking agents. After an appropriate incubation period, the sec-

TABLE II

The density of excitatory and inhibitory binding sites in the dentate nucleus

All values in pmol/mg protein (S.E.M.). Percent of cerebellar cortex is the ratio of DN to cerebellar cortex binding, an index of the relative level of each binding site. N.S., no significant binding.

Binding site	*Binding site levels*	*% cerebellar cortex*
Benzodiazepine	0.076 (0.015)	23%
$GABA_A$	0.046 (0.013)	5%
$GABA_B$	N.S.	–
NMDA		–
[^{3}H]Glutamate	N.S.	
[^{3}H]Glycine	N.S.	
[^{3}H]MK-801	N.S.	
AMPA	0.271 (0.012)	35%
Kainate	0.063 (0.004)	10%
Metabotropic	0.088 (0.010)	22%

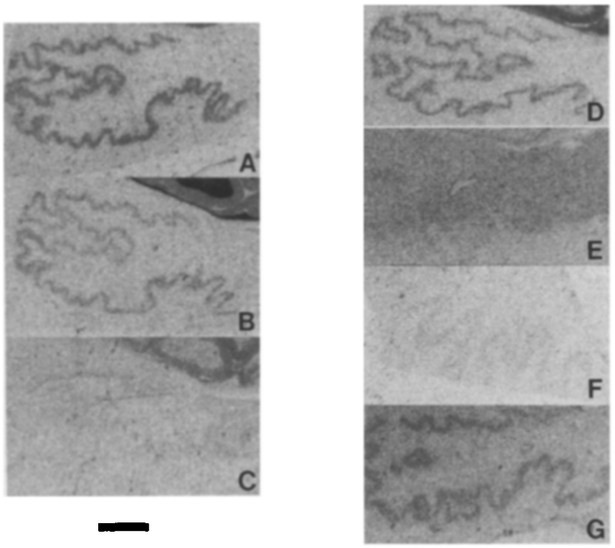

Fig. 1. Autoradiographs of the GABA and EAA binding sites in the dentate nucleus. (A) Benzodiazepine binding; (B) $GABA_A$ binding; (C) $GABA_B$ binding; (D) AMPA binding; (E) strychnine-insensitive [^{3}H]glycine binding, a measure of *N*-methyl-D-aspartate binding sites; (F) kainate binding; (G) metabotropic binding. Bar = 2 mm.

tions received 3 (GABA assays) or 4 (remaining assays) quick squirts of buffer (4 °C) followed by one (GABA assays) or two (remaining assays) quick squirts of 2.5% glutaraldehyde in acetone, and dried under a stream of hot air.

MK-801 binding was assessed with the method of Sakurai et al. (Table I)[22]. Sections were prewashed in buffer for 30 min at 4 °C, dried under a stream of cool air, and immersed in ligand solution containing 20 nM [^{3}H]MK-801 for 120 min at room temperature. Following incubation, sections were rinsed in buffer at 4 °C for 80 min, and dried under a stream of hot air.

To measure BDZ binding, sections received 3 × 10 min washes in buffer (4 °C), dried under a stream of cool air, and immersed in ligand solution for 30 min. Following incubation with ligand, sections received one quick dip in buffer (4 °C) followed by 2 × 5 min rinses in buffer (4 °C), and were dried under a stream of hot air.

Slides were then apposed to tritium sensitive film (Hyperfilm, Amersham) and exposed along with known radioactive standards for ten days to six weeks. Films were developed in Kodak D-19 and binding site density was quantitated with computer assisted densitometry using the MCID system (Imaging Research, St. Catherines, Ont.). To give a relative index of the level of binding within the DN, ligand binding in cerebellar cortex was also measured and the ratio of DN to cerebellar cortical binding was computed. Cerebellar cortex was present on all blocks of tissue used in this study and contains high levels of all GABA and EAA binding sites, allowing use of cerebellar cortical values for comparison with the DN.

Data analysis showed that only some receptor subtypes were present within the DN (Table II, Fig. 1). NMDA binding sites, whether assayed with [^{3}H]glutamate, [^{3}H]MK-801, or [^{3}H]glycine, were not found within the DN (Fig. 1, Table II). Similarly, $GABA_B$ binding sites were not found within the DN. The level of KA binding within the DN was low, less than 10% of cerebellar cortical binding. AMPA, MET, BDZ, and $GABA_A$ binding sites were readily identified within the DN (Table II, Fig. 1). AMPA, MET, and BDZ binding was at least 20% of cerebellar cortical binding levels. $GABA_A$ binding was 5% of cerebellar cortical binding, however, this apparently low level of relative binding reflects the very high livel of $GABA_A$ binding in cerebellar cortex (see Albin and Gilman[1]). BDZ binding may give a better measure of the relative level of $GABA_A$/BDZ binding sites in the DN.

Our data suggest that $GABA_A$/BDZ receptors are the primary mediators of inhibitory neurotransmission and that AMPA and MET receptors are the primary mediators of excitatory neurotransmission within the human DN. Given the similarities in connectional anatomy and cytoarchitecture among the DCN, our findings may apply also to the other nuclei of the DCN. Our GABA binding site results are similar to those documented in prior autoradiographic studies of rodent and human cerebellum[3,26]. In addition, Meinecke et al. have shown that DCN neuron perikarya and dendrites contain BDZ receptor immunoreactivity[14]. EAA binding sites in the DCN have not been studied carefully in rodents or other species. Few prior autoradiographic studies comment on EAA binding sites within the DCN. Monaghan et al. found a low, but measureable density of NMDA binding sites within the rat DCN[17], and Monaghan and Cotman found a low density of KA binding sites within the lateral deep cerebellar nucleus of the rat[16], the homologue of the primate DN. Rat DCN neurons respond to iontophoretically applied glutamate, aspartate, NMDA, and quisqualate[2,11], and responses to glutamate are partially blocked by specific antagonists of either NMDA and non-NMDA receptors[2]. These data indicate the presence of both NMDA and ionotropic-quisqualate (i.e. AMPA) receptors on DCN neurons. Our finding that the human DN appears to lack NMDA binding sites, while these binding sites/receptors are present in the DCN of rats, suggests a significant interspecies difference in the organization of EAAergic pathways within the DCN.

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