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2,4,5-Trihydroxyphenylalanine (6-hydroxy-DOPA) displaces [^{3}H]AMPA binding in rat striatum

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Excitatory amino acid (EAA) receptor-mediated events have recently been implicated in dopaminergic mechanisms of neurotoxicity. 2,4,5-Trihydroxyphenylalanine (6-hydroxy-DOPA, TOPA), the *ortho*-hydroxylated derivative of the dopamine precursor 2,4-dihydroxyphenylalanine (L-DOPA), has recently been reported to have neurotoxic properties which are blocked by CNQX, a specific antagonist of the AMPA class of non-*N*-methyl-D-aspartate (non-NMDA) EAA receptors. We report here that 6-hydroxy-DOPA is a selective displacer of [^{3}H]AMPA binding in rodent brain. 6-Hydroxy-DOPA was as potent as kainate in displacing [^{3}H]AMPA binding, with an IC_{50} value of 32 μM. Ineffective displacers of [^{3}H]AMPA binding included dopamine, 6-hydroxydopamine, L-DOPA, D-DOPA, carbidopa, DOPAC, β-methylamino-L-alanine, 2,4-dihydroxyphenylacetyl-L-asparagine, homogentisic acid, 2,4-dihydroxyphenylacetic acid, amantadine, and *threo*-DOPS. 6-Hydroxy-DOPA (100 μM) also displaced 20% of [^{3}H]kainate binding, but did not displace binding to NMDA, phencyclidine (PCP), or dopaminergic (D_1 and D_2) receptors. These data raise the possibility that 6-hydroxy-DOPA or another abnormal metabolite of L-DOPA could act as an excitotoxic agent via action at AMPA receptors. Given that non-NMDA receptors are postulated to play a role in neurotoxic events, these data provide an additional mechanism via which EAA receptor-mediated events could produce neurodegeneration in areas of brain with dopaminergic innervation.

Abnormal activation of excitatory amino acid (EAA) receptors has been implicated in the pathophysiology of various types of neurotoxic events including epilepsy [5], neuronal damage resulting from hypoxia-ischemia [15], olivopontocerebellar atrophy [13], and Alzheimer's disease [9, 11]. Huntington's disease and Parkinson's disease are neurodegenerative diseases in which dopaminergic systems or their targets are preferentially affected. EAA receptor-mediated mechanisms have recently been implicated in the pathophysiology of both Huntington's disease [4] and Parkinson's disease [8, 17]. While the regional specificity of the neuropathologic changes seen in Huntington's and Parkinson's diseases is as yet unexplained, recent observations emphasize the importance of interactions between dopaminergic and EAA systems. MK-801, a specific antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of EAA receptor, blocks methamphetamine-induced nigrostriatal toxicity [16]. In addition, several NMDA antagonists have been shown to block the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the substantia nigra [17].

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Olney et al. [10] recently reported that L-DOPA and its *ortho*-hydroxylated derivative 6-hydroxy-DOPA produced neurotoxic damage of the chick retina in a manner similar to that induced by the EAA agonists kainate and quisqualate. The non-NMDA receptor antagonist 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX) blocked neurotoxic effects of all the tested compounds, but the NMDA receptor antagonist MK-801 did not. In addition, 6-hydroxy-DOPA produced depolarizations of cultured rat hippocampal neurons which were blocked by CNQX but not by the NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV) [1, 10, 14]. These results clearly indicated a potential interaction of 6-hydroxy-DOPA and AMPA receptors. The present experiments were directed towards this question.

Autoradiographic binding assays were performed according to previously published methods (Table I). Briefly, the following materials were employed (receptor type, [^{3}H]ligand, buffer, incubation conditions, rinse time): ionotropic quisqualate/AMPA receptors, [^{3}H]AMPA, 50 mM Tris-HCl + 2.5 mM $CaCl_2$ + 30 mM KSCN pH 7.2, 45 min at 4°C, < 10 s in buffer followed by 2.5% glutaraldehyde in acetone; kainate receptors, [^{3}H]kainate, 50 mM Tris-acetate, 45 min at 4°C, < 10 s in buffer followed by 2.5% glutaraldehyde in acetone;

TABLE I

AUTORADIOGRAPHIC BINDING ASSAYS

Numbers represent percentage of control binding for each assay ± S.E.M. ($n = 4$ animals). All compounds tested at a concentration of 100 μM. n.t., not tested.

Receptor assay:	AMPA	Kainate	NMDA	PCP	D_1 Receptors	D_2 Receptors
Ligand:	[^{3}H]AMPA	[^{3}H]Kainate	[^{3}H]Glutamate	[^{3}H]MK-801	[^{3}H]SCH-23390	[^{3}H]Spiperone
Ligand concentration:	15 nM	60 nM	65 nM	5 nM	495 pM	250 pM
Specific activity (Ci/mmol):	60	58	46	22.5	83	93
Non-specific binding:	1 mM Glu	100 μM kainate	1 mM NMDA	5 μM MK-801	10 μM *cis*-flupenthixol	500 μM dopamine
6-Hydroxy-DOPA	20 ± 1	80 ± 3	109 ± 19	94 ± 6	103 ± 3	105 ± 12
Quisqualate	2 ± 1	2 ± 0	44 ± 14	n.t.	91 ± 14	107 ± 11
AMPA	1 ± 0	n.t.	n.t.	n.t.	90 ± 2	119 ± 20
BMAA	102 ± 8	89 ± 7	118 ± 29	100 ± 7	100 ± 4	99 ± 14
2,4-Dihydroxyphenylacetyl-L-asparagine	100 ± 4	101 ± 9	134 ± 28	99 ± 6	106 ± 2	95 ± 11
Homogentisic acid	106 ± 6	99 ± 6	107 ± 27	93 ± 9	119 ± 7	95 ± 14
2,4-Dihydroxyphenylacetic acid	101 ± 4	85 ± 2	95 ± 26	98 ± 9	102 ± 3	90 ± 18
Dopamine	89 ± 5	89 ± 2	110 ± 27	93 ± 5	9 ± 1	
6-OH-Dopamine	89 ± 3	90 ± 3	110 ± 29	98 ± 5	91 ± 9	75 ± 5
L-DOPA	92 ± 2	81 ± 7	90 ± 24	92 ± 4	105 ± 9	51 ± 9
D-DOPA	84 ± 10	87 ± 2	86 ± 16	99 ± 5	109 ± 5	80 ± 14
threo-DOPS	103 ± 1	100 ± 5	108 ± 28	96 ± 7	117 ± 6	78 ± 13
Amantadine	101 ± 9	79 ± 14	118 ± 25	73 ± 8	106 ± 1	69 ± 7
Carbi-DOPA	99 ± 3	92 ± 8	143 ± 43	105 ± 8	60 ± 5	51 ± 4
DOPAC	99 ± 8	86 ± 8	137 ± 33	99 ± 8	103 ± 3	94 ± 5

NMDA receptors, [^{3}H]glutamate, 50 mM Tris-acetate, 45 min at 4°C, < 10 s in buffer followed by 2.5% glutaraldehyde in acetone; phencyclidine (PCP) site within the NMDA channel, [^{3}H]MK-801, 50 mM Tris-Ac, pH 7.4, 120 min at room temperature, 80 min in cold buffer; D_1 dopamine receptors, [^{3}H]SCH-23390, 25 mM Tris-HCl + 100 mM NaCl + 1 mM $MgCl_2$ + 1 μM pargyline in 0.001% ascorbate, 150 min at room temperature, 10 min in cold buffer; D_2 dopamine receptors, [^{3}H]spiperone, 25 mM Tris-HCl + 100 mM NaCl + 1 mM $MgCl_2$ + 1 μM pargyline + 100 nM mianserin in 0.001% ascorbate, 150 min at room temperature, 10 min in cold buffer. Dried sections were placed in X-ray cassettes with appropriate radioactive standards [12] and apposed to Amersham Hyperfilm. The film was exposed to the tissue sections for 14–21 days at 4°C, then developed, fixed and dried. The optical densities of the resultant film images were determined using a commercially available computer-based image analysis system (Imaging Research, Inc., St. Catharine's, Ont. Canada). Ten to twenty-five readings were averaged from each region of interest. The amount of radioactivity was determined by a computer-generated polynomial regression analysis which compared film densities produced by the tissue sections to those produced by the radioactive standards. All data presented were analyzed densitometrically from autoradiographic images.

6-Hydroxy-DOPA (100 μM) displaced 80% of [^{3}H]AMPA binding in rat striatum (Table I). Quisqualate and AMPA (100 μM) completely displaced [^{3}H]AMPA binding, but [^{3}H]AMPA binding was displaced less than 11% by L-DOPA, *S*(−)-α-hydrazino-3,4-dihydro-α-methylbenzenepropanoic acid (carbi-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), amantadine, DL-*threo*-3,4-dihydroxyphenylserine (*threo*-DOPS), dopamine, 6-hydroxydopamine, 2,4-dihydroxyphenylacetyl-L-asparagine, homogentisic acid, or 2,4-dihydroxyphenylacetic acid. D-DOPA displaced 16% of [^{3}H]AMPA binding. 6-Hydroxy-DOPA ($IC_{50} = 32$ μM) was as potent as kainate in displacing [^{3}H]AMPA binding ($IC_{50} = 48$ μM). Other effective displacers of [^{3}H]AMPA binding included the AMPA receptor antagonist CNQX ($IC_{50} = 476$ nM), β-*N*-oxalylamino-L-alanine (BOAA) ($IC_{50} = 1.2$ μM), and quisqualate ($IC_{50} = 47$ nM).

6-Hydroxy-DOPA also displaced 20% of striatal [^{3}H]kainate binding, as did L-DOPA. 6-Hydroxy-DOPA had no effect on NMDA-sensitive [^{3}H]glutamate binding, [^{3}H]MK-801 binding, [^{3}H]SCH-23390 (D_1 receptor) binding or [^{3}H]spiperone (D_2 receptor) binding (Table I). Other structurally similar compounds, when tested at a concentration of 100 μM, failed to displace significant amounts of [^{3}H]AMPA binding. Notably, L-DOPA displaced only 9% of [^{3}H]AMPA binding, indicating that any interaction of L-DOPA at the AMPA receptor is a weak one.

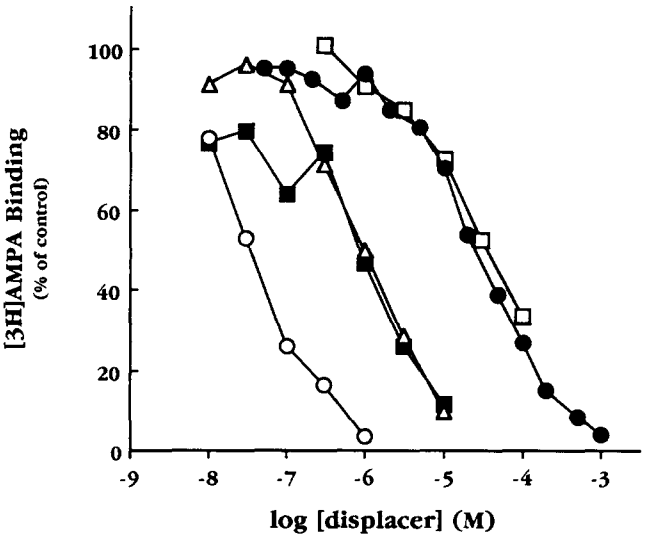

Fig. 1. Displacers of [^{3}H]AMPA binding in rat striatum: quisqualate (○); CNQX (■); BOAA (△); 6-hydroxy-DOPA (●); kainate (□). Points represent the means of data from 3 animals. Standard errors of the mean were less than 15%. Concentration of [^{3}H]AMPA was 37 nM.

6-Hydroxy-DOPA has been shown to be a potent excitant of frog spinal neurons and a potent convulsant when injected intraventricularly in rats [2]. Olney et al. [10] reported that the dopamine precursor L-DOPA and its *ortho*-hydroxylated analogue 6-hydroxy-DOPA produced neurotoxic damage of the chick retina in a fashion reminiscent of EAA-induced neurotoxicity. While 6-hydroxy-DOPA is not normally found in mammalian brain, it can be generated from L-DOPA via the action of tyrosinase [7]. It is also possible that other pathways for the abnormal hydroxylation of L-DOPA could produce 6-hydroxy-DOPA. For example, manganese ions can catalyze the formation of 6-hydroxydopamine from dopamine. Although 6-hydroxy-DOPA can be converted to the dopamine neurotoxin 6-hydroxydopamine, injections of 6-hydroxy-DOPA, which permeates the blood–brain barrier, do not lead to depletions of striatal dopamine [6]. In the present study, 6-hydroxydopamine was not an effective displacer of EAA receptor binding.

Rosenberg et al. [14] recently demonstrated that 6-hydroxy-DOPA oxidizes in solutions of physiologic pH to form a compound which is a neurotoxin and a non-NMDA receptor agonist. They concluded that the active compound was likely to be an oxidation product of 6-hydroxy-DOPA rather than 6-hydroxy-DOPA itself. Although in the present study 6-hydroxy-DOPA was dissolved in solutions containing 1 mM ascorbate and the [^{3}H]AMPA binding assay was performed at 4°C, we cannot exclude the possibility that oxidation occurred. Some degree of oxidation of 6-hydroxy-DOPA surely occurred, as evidenced by the change of solution color from clear to reddish orange. Displacement of [^{3}H]AMPA binding in our study may have then been due to the presence of an oxidized metabolite of 6-hydroxy-DOPA, rather than 6-hydroxy-DOPA itself. Since 6-hydroxy-DOPA oxidizes rapidly at physiologic pH, the present results are relevant in that a similar oxidation reaction is likely to occur in neural tissues.

Recent studies using chick retina preparations have demonstrated that 6-hydroxy-DOPA has neurotoxic properties [10, 14]. Electrophysiological responses and neurotoxic damage produced by 6-hydroxy-DOPA were blocked by the specific non-NMDA receptor EAA antagonist CNQX and not by the NMDA receptor antagonist APV, suggesting a specific involvement of non-NMDA EAA receptors [1, 10]. The present study confirms that 6-hydroxy-DOPA or a rapidly produced metabolite thereof selectively interacts with AMPA receptors and, to a lesser extent, kainate receptors. Both of these receptor types have been implicated in neurotoxic phenomena [3, 18]. Given that 6-hydroxy-DOPA can be produced by human melanocyte tyrosinase [7], the possibility is raised that 6-hydroxy-DOPA or another abnormal metabolite of the catecholaminergic synthetic pathway could act as an endogenous neurotoxin. If such a neurotoxin were selectively generated only by catecholaminergic neurons, these same neurons or their target neurons would be selectively vulnerable to EAA receptor-mediated degeneration. Taken together, these observations provide a plausible mechanism for the regional specificity of neuronal death in both Huntington's disease and Parkinson's disease.

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