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Localization of *N*-Methyl-D-Aspartate Receptors in the Rat Striatum: Effects of Specific Lesions on the [^{3}H]3-(2-Carboxypiperazin-4-yl)propyl-1-Phosphonic Acid Binding

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Abstract: The binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP), a rigid analogue of 2-amino-7-phosphonoheptanoic acid (AP7) and reported to be a selective *N*-methyl-D-aspartate (NMDA) antagonist, was studied in rat striatal membranes using a centrifugation procedure to separate bound and free radioligand. [^{3}H]CPP bound with high affinity (K_D = 272 n*M*) in a saturable, reversible, and protein concentration–dependent manner. Specific binding was suggested to involve a single class of noninteracting binding sites. The most potent [^{3}H]CPP binding inhibitors tested were CPP, L-glutamate, 2-amino-5-phosphonovalerate, and AP7. NMDA, L-aspartate, and α-aminoadipate were also shown to be efficient in inhibiting the binding, whereas quisqualate, D,L-2-amino-4-phosphonobutyrate, kainate, L-glutamate diethylester, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid were found to be essentially inactive. These data are therefore consistent with the view that [^{3}H]CPP selectively binds to NMDA receptors in the rat striatum. Lesions of intrastriatal neurons using local injections of kainic acid revealed a marked decrease in [^{3}H]CPP binding, suggesting an almost exclusively postsynaptic location of binding sites in the striatum. Conversely, bilateral lesion of corticostriatal glutamatergic fibers resulted in an increased number of [^{3}H]CPP striatal binding sites, providing evidence for a putative supersensitivity response to this striatal deafferentation. Interestingly, lesion of the nigrostriatal dopaminergic neurons using intranigral 6-hydroxydopamine injections resulted, 2–3 weeks later, in a similar increase in the number of [^{3}H]CPP striatal binding sites. These data suggest the occurrence of functional receptor-receptor interregulations at the postsynaptic level between dopaminergic and NMDA receptors in complement with the interactions occurring at the presynaptic level between glutamatergic and dopaminergic nerve terminals. **Key Words:** Glutamate receptors—3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid—Striatum—Nigrostriatal dopaminergic system. **Samuel D. et al.** Localization of *N*-methyl-D-aspartate receptors in the rat striatum: Effects of specific lesions on the [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid binding. *J. Neurochem.* **54**, 1926–1933 (1990).

3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), a rigid analogue of 2-amino-7-phosphonoheptanoic acid (AP7), was recently developed as a competitive, selective inhibitor of excitatory amino acids (EAAs) acting at the *N*-methyl-D-aspartate (NMDA) receptor subclass (Olverman et al., 1986). Indeed, on the basis of electrophysiological studies, CPP was shown to represent the most potent antagonist of the depolarizing effects of NMDA on spinal motoneurons in vitro and of NMDA-induced excitation in vivo, compared with other previously reported selective NMDA antagonists such as 2-amino-5-phosphonopentanoate (AP5) and AP7 (Davies et al., 1986; Harris et al., 1986; Childs et al., 1988). CPP has no effect on neuronal depolarization produced by quisqualate and kainate, the preferential agonists of the two other subclasses of EAA receptors (Harris et al., 1986). Moreover, CPP was shown to selectively and competitively antagonize the release of [^{3}H]acetylcholine ([^{3}H]ACh) from rat striatal slices induced by NMDA but not the release evoked by KCl (Lehmann et al., 1987). CPP was also shown to exhibit potent anticonvulsant prop-

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Abbreviations used: α-AA, α-aminoadipate; ACh, acetylcholine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP4, 2-amino-4-phosphonobutyrate; AP5, 2-amino-5-phosphonopentanoate; AP7, 2-amino-7-phosphonoheptanoate; ChAT, choline acetyltransferase; CPP, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; DOPAC, dihydroxyphenylacetic acid; EAA, excitatory amino acid; GABA, γ-aminobutyrate; GAD, glutamate decarboxylase; GDEE, glutamate diethylester; NMDA, *N*-methyl-D-aspartate; 6-OHDA, 6-hydroxydopamine.

erties and to markedly affect mechanisms of long-term potentiation in the hippocampus in vivo (Abraham and Mason, 1988).

Receptor autoradiographic studies of rat hippocampal sections have shown that CPP was able to displace [^{3}H]glutamate binding in areas rich in NMDA receptors but not in regions rich in kainate sites. In similar experiments on brain membranes, CPP did not compete with the binding of selective kainate or quisqualate site ligands such as [^{3}H]kainic acid or D,L[^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), respectively (Harris et al., 1986; Lehmann et al., 1987), but was a potent displacer of [^{3}H]AP5 binding to rat cerebral cortex membranes (Davies et al., 1986; Olverman et al., 1988). Data from [^{3}H]CPP binding studies were consistent with selective competitive antagonism at NMDA-type receptors. With autoradiography, [^{3}H]CPP binding sites showed an uneven distribution in the brain, but comparison to NMDA-displaceable [^{3}H]Glu binding site distribution suggested the existence of two subpopulations of NMDA receptors, only one of which had high affinity for [^{3}H]CPP (Olverman et al., 1986). In the same study, [^{3}H]CPP binding to synaptic membranes from rat cerebral cortex gave an apparent K_D value of 280 nM, with pharmacological properties in agreement with a selective binding to the NMDA-type receptors and not to kainate- and quisqualate-related sites.

In the striatum, where the corticostriatal afferent fibers are thought to use an EAA as a neurotransmitter (see Fonnum, 1984), electrophysiological experiments have shown in the cat that excitatory responses induced by iontophoretic applications of quisqualate and kainate resembled those evoked by electrical stimulation of the neocortex (Herrling, 1985). This is apparently not the case for excitation induced by NMDA since the synaptic excitation following cortical stimulation was not blocked by NMDA receptor antagonists. However, in vitro studies of striatal [^{3}H]ACh release evoked by NMDA application have suggested a selective contribution of NMDA-type receptors in the corticostriatal activation of ACh-containing striatal neurons (Lehmann and Scatton, 1982). Studies of dopamine release have also suggested the existence of EAA receptors located presynaptically on the dopaminergic nigrostriatal nerve terminals (Giorguieff et al., 1977; Chéramy et al., 1986; Snell and Johnson, 1986) which could be involved in the induced-activatory effects of corticostriatal neuron stimulation on striatal dopamine release (Nieoullon et al., 1978). However, even if data from [^{3}H]Glu binding experiments have suggested the existence of Glu receptors on the dopaminergic nerve terminals (Roberts et al., 1982), the subtype of the putative EAA receptors involved in this connection remains to be determined. Thus, in the striatum, the nature of EAA receptors involved in both corticostriatal transmission of excitatory neuronal effects from the cerebral cortex and cortically induced presynaptic activation of dopamine release is still unclear. In recent experiments, in an attempt to determine the location of quisqualate-type receptors in rat striatum, we studied [^{3}H]AMPA binding to synaptic membranes (Errami and Nieoullon, 1988). Lesions of intrastriatal neurons by local injection of kainic acid induced a large decrease (63%) in [^{3}H]AMPA binding 2–3 weeks later, whereas selective lesions of the nigrostriatal dopaminergic pathway by intranigral injection of 6-hydroxydopamine (6-OHDA) were without any influence. These data illustrate that quisqualate-preferring EAA receptors may be primarily associated with postsynaptic intrastriatal neurons but not located on the nigrostriatal dopaminergic nerve endings. Interestingly, lesion of the cerebral cortex induced a 30% decrease in [^{3}H]AMPA binding, suggesting that some quisqualate-type receptors may be involved in corticostriatal synaptic transmission by acting in putative autoregulatory processes. [^{3}H]Kainic acid binding studies have shown that lesions of intrastriatal neurons and cortex yielded data very similar to those obtained for [^{3}H]AMPA binding in the striatum (Henke and Cuénod, 1979). In the present study we analyzed the kinetic and pharmacological properties of [^{3}H]CPP binding to rat striatal membranes and characterized neuronal striatal distribution of the [^{3}H]CPP binding sites to further approach the functional role of NMDA-type EAA receptors. In the same experiments, glutamate decarboxylase (GAD) and choline acetyltransferase (ChAT) were measured as an index of the functional integrity of, respectively, γ-aminobutyrate (GABA)-ergic and cholinergic intrastriatal neurons particularly sensitive to local striatal lesions (see Errami and Nieoullon, 1986). Tyrosine hydroxylase activity and striatal content of dopamine and dihydroxyphenylacetic acid (DOPAC) were measured following 6-OHDA-induced degeneration of nigrostriatal neurons to assess the efficiency of lesion. The extent of the cortical lesion was determined as previously, by measuring changes in high-affinity Glu uptake into striatal synaptosomes (see Errami and Nieoullon, 1988).

MATERIALS AND METHODS

Materials

[^{3}H]CPP was purchased from NEN (Southampton, U.K.). D,L-AMPA, CPP, D,L-AP7, D,L-2-amino-4-phosphonobutyrate (D,L-AP4), kainate, and quisqualate were obtained from RBI (Natick, MA, USA); and D-AP5, 6-OHDA, quinolinate, kynurenate, ibotenate, D-α-aminoadipate (D-α-AA), NMDA, L-homocysteic acid, *cis*-2-3-piperidine dicarboxylate, L-glutamate diethylester (L-GDEE), and amino acids were from Sigma (St. Louis, MO, USA).

Striatal lesions and deafferentations and their biochemical controls

Adult female Wistar rats weighing 200–250 g were used in these experimental series. Lesions of the striatum by local injections of kainic acid, of the nigrostriatal dopaminergic neurons by intranigral injections of 6-OHDA, and of corticostriatal neurons induced by large thermocoagulation of the cerebral cortex were performed in anesthetized animals as

described previously (Errami and Nieoullon, 1986, 1988). All lesions were unilateral, except in some cases for the cortical lesions, which involved the frontoparietal cortex in both sides of the brain. Animals were allowed to recover for a survival period of 2–3 weeks and biochemical data were compared with those obtained from intact control rats.

Biochemical assessment of the extent of brain lesions was performed as previously described (Errami and Nieoullon, 1986, 1988). Tyrosine hydroxylase activity was determined on striatal homogenates using the method developed by Waymire et al. (1971), and that of GAD and ChAT following the procedures of Albers and Brady (1959) and Fonnum (1975), respectively. The contents of dopamine and DOPAC were determined using an HPLC method coupled with electrochemical detection of compounds (Dusticier and Nieoullon, 1987), and high-affinity Glu uptake in striatal synaptosomes was assayed as previously described (Kerkerian and Nieoullon, 1988). Protein content of each sample was measured using the method of Lowry et al. (1951) with bovine serum albumin as standard.

[^{3}H]CPP binding assay

Striatal membranes were obtained from animals killed by decapitation. Brains were quickly removed and dissected at 4°C. Striatal tissue was homogenized, using a Potter, in 25 vol (wt/vol) of an ice-cold solution containing $CaCl_2$ 1.2 m*M*, $MgSO_4$ 1.2 m*M*, Na_2PO_4 1.2 m*M*, KCl 5 m*M*, NaCl 140 m*M*, D-glucose 10 m*M*, and Tris buffer 15.6 m*M* at pH 7.4, and the homogenate was centrifuged at 500 *g* for 5 min at 4°C. The supernatant was recentrifuged at 4°C for 30 min at 40,000 *g* and the resultant P_2 pellet lysed with 10 ml glass-distilled water. The membranes were then incubated for 30 min at 37°C to release endogenous EAAs and centrifuged one time more for 30 min at 40,000 *g*. The resulting pellet was dispersed in 5 ml of a 50 m*M* Tris-acetate buffer, pH 7.4, and frozen at −70°C until use. On the day of the assay, membranes were washed three more times by successive centrifugations for 30 min at 40,000 *g*. After each centrifugation, pellets were suspended in 5 ml of the Tris-acetate-buffered solution and immediately frozen. At the end of the last washing procedure, membranes were incubated at 37°C for 30 min at a concentration of 0.10–0.15 mg protein/100 μl of the Tris-acetate buffer with either 100 n*M* [^{3}H]CPP (specific activity 30.7 Ci/mmol) or regularly increasing concentrations of the labeled compound from 5 to 750 n*M* for saturation binding experiments. In pharmacological experiments, [^{3}H]CPP (100 n*M*) was incubated in the presence or absence of various concentrations of compounds under test. L-Glu (1 m*M*) or unlabeled CPP (100 μ*M*) was used to define nonspecific binding. [^{3}H]CPP binding was stopped by centrifugation (10 min, 15,000 *g*) and the pellet routinely washed in 1 ml of the Tris-acetate buffer. After this washing procedure, the pellet was resuspended in 1% Triton X-100 and the amount of radioactivity measured by liquid scintillation spectrometry. Specific binding was determined by subtracting the nonspecific binding component for each concentration of the radiolabeled ligand from total binding. In animals with lesion, [^{3}H]CPP binding was determined either at the concentration of 100 n*M* or in saturation procedure.

Data analysis

When [^{3}H]CPP binding was measured at the concentration of 100 n*M*, results were usually expressed as mean ± SEM values of data obtained in quadruplicate in series of at least six animals. Statistical comparisons of data obtained in animals with lesions and in control groups were performed using Student's *t* test. For each biochemical assay, the reaction was previously shown to be linear with time and tissue amount in the range used in the experiments. Data from saturation binding experiments were analyzed using an iterative curve-fitting computer program (Vindimian et al., 1983). IC_{50} values obtained in pharmacological experiments were determined graphically and K_i values calculated from the equation $K_i = IC_{50}/(1 + IC_{50}/L)$, where L is the [^{3}H]CPP concentration.

RESULTS

Biochemical characteristics of [^{3}H]CPP binding to rat striatal membranes

[^{3}H]CPP specific binding at 100 n*M* represented ~60% of total binding at equilibrium. However, the washing procedure of the pellet, by resuspension, after centrifugation, which stopped the incubation, decreased nonspecific binding but also led to highly decreased specific binding and nonspecific/specific binding ratio. An increase in medium osmolarity by 0.5 *M* sucrose had no effect on [^{3}H]CPP specific binding. The specific binding of [^{3}H]CPP was shown to increase rapidly with incubation time to reach a plateau after 30 min. Specific binding was also rapidly reversed by addition of 1 m*M* L-Glu or 100 μ*M* CPP (data not shown). Specific [^{3}H]CPP binding was found to be dependent on the protein concentration in the range of 0.05–0.15 mg protein/100 μl (data not shown). In saturation experiments, when the membrane preparation was incubated with increasing concentrations of the radiolabeled ligand from 5 to 750 n*M*, specific binding was shown to be saturable (Fig. 1). Nonspecific binding (not displaceable in the presence of L-Glu or CPP in excess)

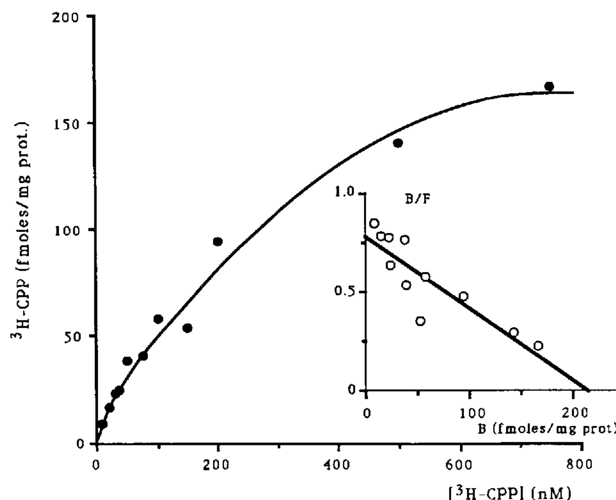

FIG. 1. Saturation isotherm and Scatchard plot (**inset**) of [^{3}H]CPP binding to rat striatal membranes. Membranes were incubated for 30 min at 37°C with increasing concentrations of [^{3}H]CPP from 5 to 750 n*M*. Specific binding was obtained by subtracting nonspecific binding measured in the presence of 1 m*M* L-Glu from total binding. Values were obtained from analysis performed in quadruplicate in 16 experiments. B and F represent bound and free concentrations of the labeled ligand, respectively.

was linear. Scatchard analysis revealed, in the range of concentration from 5 to 750 nM of the radiolabeled compound, a single binding site population with a K_D value of 272 nM and an apparent B_{max} value of 213 fmol/mg protein (Fig. 1). The Hill coefficient of the saturation curve was close to 1 (1.02), suggesting the absence of cooperative interactions between the binding sites.

Pharmacological characterization of [^{3}H]CPP binding sites on rat striatal membranes

The stereoselectivity of the specific binding of [^{3}H]CPP at 100 nM was shown by the marked inhibition induced by L-Glu and L-aspartate at 100 μM compared with weaker effects produced by the D-enantiomer of these two putative endogenous ligands (Table 1). In similar experiments where various agonists and antagonists of the different subclasses of the EAA receptors were introduced into the incubation medium at the concentration of 100 μM to compete with the [^{3}H]CPP binding at 100 nM, unlabeled CPP and L-Glu represented the most potent inhibitors. Quisqualate and kainate, respectively, showed 48 and 32% inhibition, whereas NMDA was the most effective in inhibiting [^{3}H]CPP binding (82% inhibition). This great selective efficiency of an NMDA-type receptor agonist to displace [^{3}H]CPP binding was also illustrated by the very weak influence of AMPA, whereas ibotenate had moderate influence, and especially by the marked inhibition produced by the application of the known potent competitive antagonists of NMDA receptors: AP5, AP7, and D-α-AA. γ-Glutamylglycine, *cis*-2,3-piperidine dicarboxylate, and D,L-AP4 represented moderate to weak inhibitors of [^{3}H]CPP binding. Finally, kynurenate, quinolinate, and L-GDEE could be considered as weak to very weak inhibitors.

TABLE 1. *Effects of various EAA-related compounds on specific [^{3}H]CPP binding to rat striatal membranes*

Compound	Percentage inhibition at 100 μM
CPP	96
L-Glu	96
D-AP5	90
NMDA	82
L-Asp	82
D,L-AP7	81
D-α-AA	76
L-Homocysteic acid	65
D-Asp	60
D-Glu	59
Ibotenate	53
γ-D-Glutamylglycine	50
Quisqualate	48
cis-2,3-Piperidine dicarboxylate	47
D,L-AP4	46
Kynurenate	43
Quinolinate	37
Kainate	32
L-GDEE	20
D,L-AMPA	15

Compounds were assayed in quadruplicate at a final concentration of 100 μM using 100 nM radiolabeled ligand. Data are expressed as the average of at least six experiments (SD < 15% variation).

Competition curves (Fig. 2) showed that CPP itself, L-Glu, and AP5 were the most potent displacers of striatal [^{3}H]CPP binding. The K_i value (Table 2) calculated for CPP was slightly greater than the K_D value for [^{3}H]CPP binding (320 vs. 272 nM). Concerning the other inhibitors tested, L-Glu and AP5 showed similar abilities to displace the binding. AP7, NMDA, L-aspartate, D-α-AA, and L-homocysteate were shown to be ~10–20 times less effective than CPP, L-Glu, and AP5 in displacing the [^{3}H]CPP binding, and quisqualate ~1,000 times less effective. D,L-AP4 and especially kainate appeared to be very weak displacers.

Effects of striatal lesions and deafferentations on [^{3}H]CPP striatal binding

Kainic acid injected in the striatum induced extensive destruction of striatal neuronal populations as shown by the dramatic decrease in ChAT and GAD measured locally (Table 3). In that situation, [^{3}H]CPP binding measured at the concentration of 100 nM was extremely low, representing only 17.1% of control values estimated in intact animals. In contrast, under similar binding assay conditions, a large unilateral cortical lesion induced after 2–3 weeks a slight increase in [^{3}H]CPP striatal binding (+13%), although a large decrease in high-affinity [^{3}H]Glu uptake in synaptosomes reflected extensive damage of corticostriatal fibers (Fig. 3; Table 3). This increase in [^{3}H]CPP binding became highly significant after bilateral lesions (+35%; $p < 0.01$). Kinetic analysis of this response to a bilateral cortical lesion showed that the apparent increase in [^{3}H]CPP striatal binding resulted selectively from a significant increase in B_{max} (+52.1%) without any change in affinity of the binding sites for [^{3}H]CPP. Values of K_D and B_{max} were 248 nM and 324 fmol/mg protein, respectively, in animals with bilateral cortical lesions (Fig. 4). Interestingly, extensive unilateral lesion of the dopaminergic afferent pathway to the striatum (Fig. 3; Table 3) also resulted after 2- to 3-week survival periods in an increase (+42%; $p < 0.01$) in [^{3}H]CPP striatal binding measured at the concentration of 100 nM. This increase was shown in saturation curve analysis to be, as in the case of cortical lesion, concomitant with a selective augmentation in B_{max} (+59.6%) without any change in K_D (Fig. 5). In animals with the 6-OHDA lesion, K_D and B_{max} were 226 nM and 340 fmol/mg protein, respectively. After cortical deafferentation or lesion of the nigrostriatal dopaminergic system, saturation isotherms showed Hill coefficients of 0.96 and 1.01, respectively.

DISCUSSION

Using a centrifugation procedure to separate bound and free radioligand, the binding of [^{3}H]CPP to striatal membranes from intact control rats was found to be reversible, highly selective, and saturable. Saturation

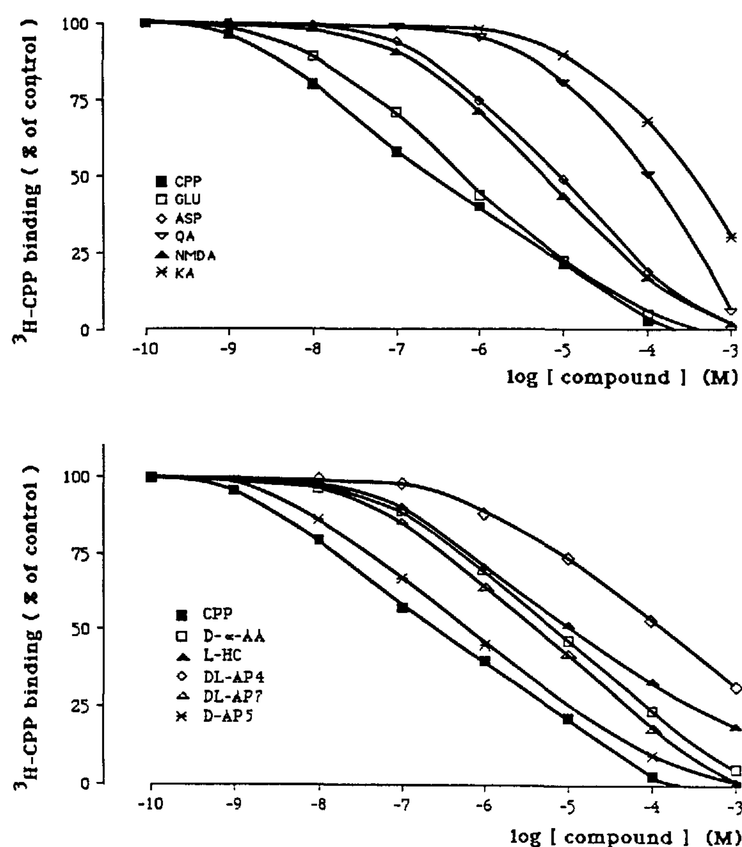

FIG. 2. Pharmacology of [^{3}H]CPP binding in rat striatal membranes. Striatal membranes were incubated for 30 min at 37°C in the presence of 100 n*M* [^{3}H]CPP with and without additional concentrations from 10^{-10} to 10^{-3} *M* of CPP, D-AP5, D,L-AP7, D,L-AP4, D-α-AA, NMDA, quisqualate (QA), kainate (KA), L-homocysteic acid (L-HC), L-Asp, and L-Glu. Each curve represents data from at least three separate experiments performed in quadruplicate at each concentration.

isotherm analysis suggested the existence of a single class of binding sites with a K_D value of 272 n*M*. This value is strictly in the range of previously published data on rat cerebral cortex membranes showing K_D values of 280 n*M* (Olverman et al., 1986; Murphy et al., 1987). Moreover, the value of the Hill coefficient, calculated from the saturation data, is in agreement with the hypothesis of the presence of a single population of binding sites without any cooperative interactions between them.

TABLE 2. *Pharmacology of specific [^{3}H]CPP binding to rat striatal membranes*

Compound	K_i (n*M*)
CPP	320
L-Glu	510
D-AP5	510
D,L-AP7	4,800
NMDA	5,800
L-Asp	6,100
D-α-AA	6,100
L-Homocysteate	9,600
Quisqualate	64,000
D,L-AP4	128,000
Kainate	321,000

[^{3}H]CPP 100 n*M* was incubated in the presence of various agonists and antagonists of EAAs at concentrations from 10^{-10} to 10^{-3} *M* for 30 min at 37°C as described in Fig. 2.

The ability of several EAA receptor agonists and antagonists and EAA-related compounds to inhibit striatal [^{3}H]CPP binding was found to be very similar to that observed by Olverman et al. (1986) and Murphy et al. (1987). The most potent inhibitors of [^{3}H]CPP binding were represented by L-Glu and NMDA agonists and antagonists such as NMDA itself, AP5, AP7, and D-α-AA. Quisqualate and kainate receptor agonists were shown to be essentially inactive. GDEE, which in vivo antagonizes the EAA transmission, was shown here to be without effect on [^{3}H]CPP binding, whereas this compound was reported by Murphy et al. (1987), in the same type of experiment, to be more effective. This was also apparently the case for D-α-AA and L-homocysteate. AP4, which might depress the EAA transmission by acting on putative autoreceptor mechanisms, was also essentially without any effect on [^{3}H]CPP binding. Taken together, these data reinforce the evidence that CPP is a competitive selective inhibitor of EAA acting at the NMDA receptor subtype (Davies et al., 1986; Harris et al., 1986; Olverman et al., 1986; Lehmann et al., 1987; Murphy et al., 1987; Childs et al., 1988). Interestingly, homocysteate, which has been proposed as a putative endogenous EAA acting in the brain at the NMDA receptor subtype (Do et al., 1988; Lehmann et al., 1988), was shown to be as

TABLE 3. *Changes in striatal biochemical markers measured after 2- to 3-week survival periods in animals subjected to intrastriatal kainic acid (KA) injections, bilateral lesion of cerebral cortex, or intranigral injection of 6-OHDA*

	Control	Lesion	Percentage of control
KA striatal lesion			
ChAT (nmol/min/mg prot.)	150.1 ± 5.4	28.3 ± 2.0[a]	18.9
GAD (nmol/min/mg prot.)	1.6 ± 0.1	0.42 ± 0.1[a]	26.4
Cortical lesion (bilateral)			
High-affinity Glu uptake (nmol/min/mg prot.)	112.4 ± 10.1	53.9 ± 6.1[a]	47.9
6-OHDA lesion			
Dopamine (μg/g prot.)	8.30 ± 0.81	1.10 ± 0.05[a]	13.1
DOPAC (μg/g prot.)	0.85 ± 0.07	0.18 ± 0.09[a]	20.9

Data are means ± SEM of values obtained in triplicate from series of at least six animals.
[a] p at least <0.01 by Student's t test.

active as AP7, D-α-AA, L-aspartate, and NMDA itself in displacing [^{3}H]CPP binding.

In the striatum, the [^{3}H]CPP binding sites appeared to be almost exclusively located on postsynaptic structures sensitive to kainic acid–induced lesions of the structure, such as, for example, GABA and cholinergic neurons (see Errami and Nieoullon, 1986). Indeed, about an 83% decrease in [^{3}H]CPP binding was found, suggesting that the putative NMDA subtype of EAA receptors labeled by [^{3}H]CPP was essentially destroyed by the lesion. Similar experiments were previously performed by us to localize in the striatum the quisqualate-type receptor subclass using [^{3}H]AMPA and quisqualate-sensitive [^{3}H]Glu binding. [^{3}H]CPP binding sites were shown here more sensitive to the striatal lesion than [^{3}H]AMPA or [^{3}H]Glu binding sites, which were reduced by only 63 and ~40%, respectively (Errami and Nieoullon, 1986, 1988). In the same type of experiments, [^{3}H]kainic acid binding was previously reported to be decreased by 40% (Henke and Cuénod, 1979). Interestingly, EAA receptor losses in putamen from patients with Huntington's disease, which is characterized by extensive destruction of the striatal area, showed decreases very similar to these found here for NMDA and previously for quisqualate receptors (93 and 67%, respectively). This reinforces the idea of quite complete localization of NMDA receptors on neuronal striatal elements, whereas for quisqualate receptors this localization is only partly postsynaptic (Young et al., 1988).

The primarily postsynaptic position of [^{3}H]CPP binding sites in the striatum was confirmed by the effect of removal of corticostriatal input, which induced an increase in the number of binding sites. Indeed, such

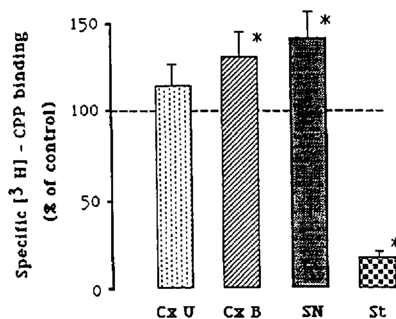

FIG. 3. Effects of specific lesions on striatal binding of [^{3}H]CPP. [^{3}H]CPP binding was measured at the concentration of 100 nM for 30 min at 37°C in animals subjected 2–3 weeks before to unilateral (CxU) or bilateral (CxB) decortication, 6-OHDA intranigral injection-induced degeneration of nigrostriatal dopaminergic neurons (SN), or kainic acid intrastriatal injection (St). Data are means ± SEM of values obtained in at least six independent experiments. Statistical significance was determined by Student's t test by comparison with the binding measured from striata in intact control animals. *p at least <0.01.

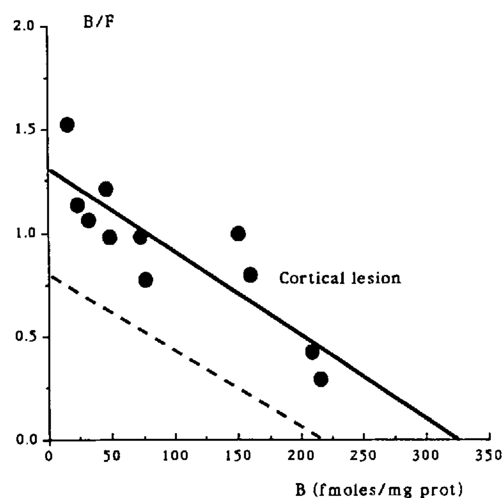

FIG. 4. Scatchard plot of [^{3}H]CPP binding to striatal membranes obtained from rats subjected 2–3 weeks before to bilateral lesion of the cerebral cortex. Membranes were incubated for 30 min at 37°C with increasing concentrations of [^{3}H]CPP from 5 to 750 nM. Specific binding data were obtained as described in Fig. 1 from series of at least six animals. The dotted line shows the data obtained from intact control animals (see Fig. 1).

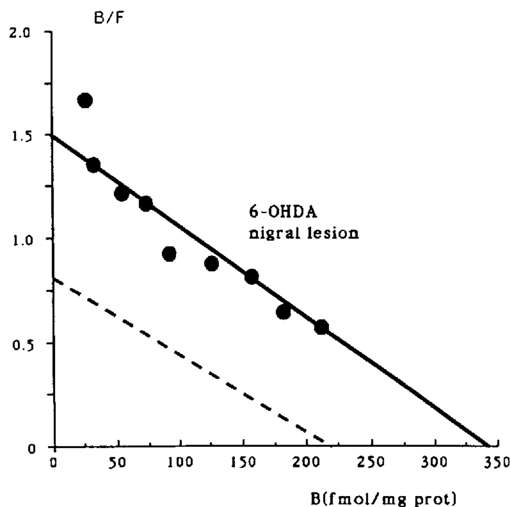

FIG. 5. Scatchard plot of [^{3}H]CPP binding to membranes from striatum in rats subjected 2–3 weeks before to a unilateral injection of 6-OHDA in the substantia nigra. Data were obtained as described in Figs. 1 and 4 from series of at least six animals.

an increase can be considered as a supersensitivity response to the glutamatergic deafferentation and further reinforces the idea that [^{3}H]CPP binds to functional postsynaptic NMDA receptor sites. Interestingly, for [^{3}H]AMPA and [^{3}H]kainic acid binding studies, the same experiment led to a decrease in apparent binding, which has suggested a presynaptic localization of subpopulations of quisqualate- and kainate-preferring receptors on corticostriatal glutamatergic nerve endings and thus their contribution to putative autoregulatory processes (Henke and Cuénod, 1979; Errami and Nieoullon, 1986).

Finally, extensive lesions of the nigrostriatal dopaminergic pathway induced a surprisingly marked increase in the number of [^{3}H]CPP binding sites, while this experiment was undertaken to evidence a possible presynaptic location of NMDA receptors on the dopaminergic endings. Data from the present experiments are not in agreement with this hypothesis. Consequently, the NMDA receptors could be involved rather in the regulation of ACh (Lehmann and Scatton, 1982) and GABA (Pin et al., 1988) release from striatal neurons than in the direct control of dopamine release from nigrostriatal nerve terminals. Indeed, such NMDA receptor activation on basal ganglia slices in vitro failed to alter dopamine release (Boldry and Uretsky, 1988). However, the present data actually show that lack of stimulation of dopaminergic receptors and/or the consequent development of their supersensitivity mechanism induced an increase in the number of [^{3}H]CPP striatal binding sites. As a first hypothesis to explain this lesion-induced response, we can propose that a default in dopaminergic receptor activation would have contributed to suppress a regulatory inhibitory influence on the neuronal NMDA receptor cellular expression. Such a mechanism would, for example, be linked to changes in second messenger mechanisms involving cyclic AMP production and protein phosphorylation, since Nestler et al. (1984) have proposed a modulatory role of dopamine receptor activation on responsiveness of neurons to Glu. Moreover, these data would suggest the occurrence of a heteroregulation of NMDA-preferring EAA receptors by dopaminergic receptors by mechanisms that remain to be determined. The increase in [^{3}H]CPP binding sites likely representing the response of NMDA subtype receptors after the dopaminergic deafferentation of the striatum can also be of indirect origin, involving modifications of Glu transmission at the presynaptic level. Indeed, increase in GABAergic transmission consequent to lack of striatal dopaminergic receptor activation is suggested to inhibit presynaptically the EAA release (see Scatton, 1987). In that situation, one can expect that, as in the case of lesion of corticostriatal glutamatergic fibers, failure in Glu release consequently induces a postsynaptic increase in NMDA receptor number.

In conclusion, the results of the present study essentially showed that [^{3}H]CPP binds to physiologically active NMDA receptors. These receptors, almost exclusively postsynaptic in the rat striatum, are sensitive to changes in activity of the corticostriatal pathway. This relationship may represent a natural mechanism regulating corticostriatal transmission. More interestingly, these receptors also appeared to be functionally linked to the striatal release of dopamine. This postsynaptic mechanism will contribute to the complex reciprocal interactions linking the two main afferent pathways to the striatum at presynaptic and postsynaptic levels (Kerkerian and Nieoullon, 1988).

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