Pharmacology Biochemistry & Behavior, Vol. 33, pp. 175–179. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Motor Effects of Intracaudate Injection of Excitatory Amino Acids

EUGENE TOTH AND ABEL LAJTHA

The Nathan S. Kline Institute for Psychiatric Research
Center for Neurochemistry, Ward's Island, New York, NY 10035

Received 14 December 1987

TOTH, E. AND A. LAJTHA. *Motor effects of intracaudate injection of excitatory amino acids.* PHARMACOL BIOCHEM BEHAV **33**(1) 175–179, 1989.—In a study of the role of excitatory amino acid receptors in movement disorders, the effect of the injection of glutamate (Glu), aspartate (Asp), N-methyl-D-aspartate (NMDA), quisqualate (Qu), or kainate (K) into the rat striatum was investigated. Rats were microinjected unilaterally through chronically implanted guide cannulas and their motor behavior was recorded. After 10–25 min L-Glu produced reversible periodic choreiform movements lasting 5–10 sec and contraversive rotation lasting 1–2 min. Both episodes were repeated every 2–3 min: the duration of motor effects was 60–80 min. L-Asp had an effect similar to that of L-Glu and in addition produced barrel rolling. The L-isomers of both Glu and Asp were active and the D-isomers were inactive. NMDA, Qu, and K were more potent than Glu or Asp. Each produced effects similar to that of Glu, and in addition NMDA and K produced wet-dog-shakes and masticatory movements. The motor behavior produced by Qu was identical to that of Glu, but it lasted longer. The motor effects of L-Glu were blocked by L-glutamic acid diethyl ester (GDEE) and by a larger sedative dose of 2-amino-5-phosphonopentanoic acid (AP5), but not by haloperidol, GABA, glycine (Gly), or a smaller nonsedative dose of AP5. The results suggest that the motor effects of L-Glu were produced by activation of the Qu-type (glutamatergic) receptors, not involving the dopamine and GABA systems. However, activation of the K-type receptors by L-Glu cannot be ruled out.

Excitatory amino acids Caudate-putamen Motor behavior

STUDIES in which ablation or undercutting of the frontal cortex resulted in a reduction in uptake and content of glutamate in the neostriatum of rats indicate that glutamate is the transmitter released by cortical fibers terminating in the striatum (5, 8, 15, 16, 18). Autoradiographic studies indicated that the glutamatergic pathway originates from layer 5 pyramidal cells in the cortex (30) and terminates in the striatum (24). Electrophysiological studies also support the glutamatergic nature of the corticostriatal tract. It was shown that glutamic acid diethyl ester (GDEE) antagonizes the excitation of striatal neurons induced by iontophoretic application of glutamic acid (29). Release of glutamic acid from the striatum in response to stimulation of the cortex was shown (14).

Glutamic acid increases the release of dopamine (13,23) and dopaminergic agonists decrease the release of glutamic acid (20,25) in striatal slices. Since dopamine regulates motor function (2,7) in the striatum, this reciprocal regulation between glutamate and dopamine indicates that glutamate also functions in motor regulation. In animals, glutamate analogues produce striatal destruction similar to that in Huntington's disease (HD) (4,17), and in the brain of HD patients the activity of glutamine synthetase is greatly reduced (3).

Since glutamic acid increases the release of dopamine in the striatum, an area involved in movement disorders (19), as part of a study of the action of glutamate on motor function we investigated the effect of excessive stimulation of striatal glutamatergic receptors on motor function by microinjecting various excitatory amino acids into freely moving rats. We compared the motor effects of L-glutamate to those of various excitatory amino acids, that bind to different subtypes of glutamatergic receptors, and we determined whether dopamine is involved in mediation of the motor effects of glutamate.

METHOD

Animals

Male Wistar rats (280–320 g), bred in our animal facility, were kept on a 12-hr light/dark cycle, fed standard diet, and given water ad lib.

Chemicals

Glutamic acid, aspartic acid, GABA, glycine, L-glutamic acid diethyl ester, haloperidol, and Evans blue were obtained from Sigma Chemical Co., St. Louis, MO. Glutamate analogues were from Research Biochemicals Inc., Natick, MA.

Stereotactic Implantation of the Guide Cannula

The surgery was performed under chloral hydrate (350 mg/kg) anesthesia. A 26-gauge stainless steel guide cannula (Plastic Products Co., Roanoke, VA) was implanted unilaterally 1 mm above the site of injection according to the coordinates of system B of the Pellegrino rat brain atlas (22) with a Kopf stereotaxic instrument. The cannula was fixed with 4 mounting screws (2 mm) and cranioplastic cement. The coordinates for the tip of the cannula were the following: 2.4 mm anterior to the bregma, 3.0 mm lateral to the midline, and 5.0 mm ventral to the surface of the skull. The incisor bar was set 5.0 mm above the interaural line.

TABLE 1

EFFECT OF INTRACAUDATE INJECTION OF EXCITATORY AMINO ACIDS

Compounds	Dose μmol/kg	N	Effect	Onset Min	Duration Min
NaCl	3.5	3	—	—	—
L-Glutamate	0.35	4	—	—	—
	0.70	5	Choreiform movements of head and forelimbs and rotation	23 ± 5	54 ± 16
	1.75	8	Choreiform movements of head, forelimbs, and trunk and rotation	14 ± 5	78 ± 16
D-Glutamate	1.75	5	Increased grooming and rearing	15 ± 7	58 ± 23
L-Aspartate	0.35	3	—	—	—
	0.70	5	—	—	—
	1.75	6	Choreiform movements of head and forelimbs and rotation; barrel rolling	23 ± 5	57 ± 20
D-Aspartate	1.75	4	—	—	—
N-Methyl-D-Aspartate	0.035	4	Wet shakes, masticatory movements	12 ± 4	86 ± 15
	0.07	4	Choreiform movements of head and forelimbs and rotation; barrel rolling, and masticatory movements	37 ± 4	168 ± 36
Quisqualate	0.035	3	—	—	—
	0.175	4	Choreiform movements of head and forelimbs and rotation	14 ± 3	120 ± 15
Kainate	0.0004	3	—	—	—
	0.0007	4	Choreiform movements of head and forelimbs and rotation	3 ± 1	102 ± 20
	0.0025	3	Choreiform movements of head and forelimbs and rotation; masticatory movements, wet shakes, and salivation	3 ± 2	240 ± 32

Rats were injected in the right caudate-putamen through chronically implanted guide cannulas. After injection they were placed in transparent bins and their motor behaviors were recorded. (N) is the number of rats tested. The values are the means of the times of onset and duration of motor effects ± S.D.

The skull landmark bregma was taken as the zero reference point.

Injection and Monitoring of Motor Functions

Seven days after surgery the unrestricted rats were injected with one of the excitatory amino acids and/or other compounds (pH 7.4) in 1.0 μl in 4 min. The injection was performed through an internal cannula (Plastic Products Co.) attached to a microsyringe. The internal cannula was kept in place for 1 min after injection. After injection the rats were placed in a 50 × 50 × 40 cm transparent bin and their motor behavior was observed visually and recorded. The animals were observed for an additional 60 min after the last motor effect.

Verification of the Site of Injection

The site of injection was verified in most of the experimental animals. The rats were injected with an overdose of chloral hydrate (900 mg/kg) IP, then perfused intracardially with isotonic saline followed by 10% formalin. The brain was cut into 100-μ coronal sections and stained with neutral red, and the site of injection was ascertained with the aid of the stereotaxic atlas. The sites of injection were at the middle ±0.5 mm of the anterior part of the striatum. To estimate the spread of injected solution in the caudate-putamen, 1.0 μl of Evans Blue was injected. The dye spread nearly spherically within a 0.8 to 0.9 mm radius around the site of the injection.

RESULTS

The injection of L-glutamate (Glu) in the right caudate-putamen of rats produced reversible periodic choreiform movements of head, forelimbs, and trunk and contraversive rotation.

TABLE 2

EFFECT OF DIFFERENT COMPOUNDS ON GLUTAMATE-INDUCED MOTOR BEHAVIOR IN THE CAUDATE-PUTAMEN

Compounds	Mode of Administration	Dose μmol/kg	N	Choreiform Movement and Rotation Onset (min)	Duration (min)
L-Glutamate	IC	1.75	8	14 ± 5	78 ± 16
L-Glutamic acid diethyl ester	IC 70 min before Glu	1.75	3	—	—
L-Glutamic acid diethyl ester	IP 75 min before Glu	2.5	4	—	—
L-Glutamic acid diethyl ester	coinjection	1.4	3	—	—
2-Amino-5-phosphonopentanoic acid	IC 10 min before Glu	0.035	4	28 ± 3	30 ± 6
2-Amino-5-phosphonopentanoic acid	IC 10 min before Glu	0.28	3	—	—
2-Amino-5-phosphonopentanoic acid	coinjection	0.28	3	—	—
GABA	coinjection	2.1	5	26 ± 5	40 ± 25
Glycine	IC 30 min before Glu	1.75	3	24 ± 12*	16 ± 6
Haloperidol	IP 30 min before Glu	13.0	3	29 ± 5	84 ± 25
Haloperidol	IC 30 min before Glu	0.026	3	20 ± 5	56 ± 10

Test compounds were pre- or coinjected with 1.75 μmol/kg L-Glu. The preinjections were IP or IC (caudate-putamen) 30, 70, or 75 min before the intracaudate administration of L-Glu. (N) is the number of animals tested. The values are the means of the times of onset and duration of motor effects ± S.D. *$p>0.05$.

The choreiform movements of the contralateral forelimb were more frequent and stronger than those of the ipsilateral. These effects in motor behavior started about 20 min after the injection of L-Glu and lasted 50–80 min (Table 1). The choreiform movements lasted for 5–10 sec and the rotation for 1–2 min. In the rotation the rats made tight head-to-tail circles at a rate of 10–20 circles per min. Both episodes were repeated every 2–5 min. The injection of D-glutamate at similar doses did not produce choreiform movements or rotation, although we observed more frequent grooming and rearing. The effect of L-glutamate was dose related: 0.35 μmol/kg had no effect, 0.70 μmol/kg induced choreiform movements of head and forelimbs and rotation, and the 1.75 μmol/kg dose in addition to the choreiform movements of head and forelimbs and rotation, produced choreiform movements of the trunk. The largest dose of L-glutamate reduced the latency of onset of the behavioral effect and produced a longer effect. Between the choreiform and rotational episodes the rats showed stereotypic behaviors also, such as excessive body grooming and perioral movements. The injection of L-glutamate in the midbrain reticular formation produced no motor effect.

L-Aspartate, like L-glutamate, induced choreiform movements of head and forelimbs and contraversive rotation, and in addition produced barrel rolling. The L-aspartate was less potent than L-glutamate, and its D-isomer was inactive at similar doses. N-Methyl-D-aspartate (NMDA) was more potent than L-glutamate and L-aspartate, and induced additional motor behavior. Besides the choreiform movements, rotation, and barrel rolling, NMDA produced strong masticatory movements and wet-dog-shakes (WDS). The WDS occurred in repeated 5–10 bursts that shook the whole body between the fore- and hind-legs. The time of onset of the NMDA effect was similar to that of L-glutamate and L-aspartate, but the duration of the effect was twice as long (168 min) after a larger dose. Quisqualate (Qu) was slightly less potent than NMDA. A 0.035 μmol/kg dose produced no effect, but at 0.175 μmol/kg it produced, like glutamate, only choreiform movements and rotation. The time of onset of effect was 14 min, similar to that of L-Glu, but the duration of the effect was longer. Kainate (K) was even more potent than NMDA. It had the shortest latency and the longest duration of effect. It also produced choreiform movements and rotation, and in addition to these effects, the larger dose produced masticatory movements, WDS, and salivation.

The motor effect of L-Glu was blocked by glutamic acid diethyl ester (GDEE) given by IC or IP preinjection or coinjection with L-Glu. 2-Amino-5-phosphonopentanoic acid (AP5) in a nonsedative dose of 0.035 μmol/kg did not block the effect of L-Glu; a dose of 0.28 μmol/kg preinjected IC or coinjected blocked the motor effect of L-Glu, but it produced sedation. GABA, Gly, or haloperidol (HA), pre- or coinjected, did not block the motor effect of L-Glu, but Gly reduced the duration of Glu effect (Table 2). The intracaudate injection of GDEE, GABA, Gly, HA, or AP5 (0.035 μmol/kg) had no effect. AP5 (0.28 μmol/kg) and HA 13.0 μmol/kg IP both produced sedation.

DISCUSSION

These results indicate that overstimulation of striatal EAA receptors results in abnormal motor function in rats. The effect of Glu and Asp, the two possible endogenous ligands of EAA receptors, appeared to be stereospecific. Only L-Glu and L-Asp produced motor effects; the D-isomers were inactive at similar doses (Table 1). The similarities and differences in the motor effects of the five EAA indicate that they activate both identical and different receptors in the striatum. That NMDA, Qu, and K have longer motor effects than those of L-Glu and L-Asp is probably due to the lack of specific transport systems to remove them from the vicinity of the EAA receptors.

The relatively short onset and duration of motor effects produced by L-Glu and L-Asp suggest that these effects are due directly to the stimulation of the EAA receptors and not to neuronal loss. It was reported that 21 days after the injection of 0.25 μmol/kg L-Glu in the striatum 30% of the neuronal population was lost, and that at this dose no behavioral effect was produced (21). However, there are other transmitters in the striatum, where Glu was shown to increase the release of DA (13,23) and to increase the accumulation of GABA (12). The EAA analogs were found to release acetylcholine in striatal slices (26). Since Glu affects the function of other neurotransmitters, it is possible that some of these transmitters mediate the Glu-induced motor effect. The difference between Glu- and Asp-induced motor effects is probably due to the fact that they bind to separate receptors also (10). The lack of motor effects of D-Glu and D-Asp at doses similar to those of their L-isomers may be explained by the higher IC_{50} values for the D-isomers (9).

Kainic acid caused neuronal damage after either local or systemic administration (27,28). It is possible that some of the observed motor effects of K were due to neuronal destruction. K produced convulsions after either systemic or unilateral amygdaloid injection (1,31). Unilateral injection of K in the striatum at the dose levels we used did not produce generalized convulsions.

The blocking of the motor effect of L-Glu by GDEE (Qu antagonist) and not by a nonsedative dose of AP5 (NMDA antagonist), and the identical motor behavior induced by L-Glu and by Qu, suggest that the motor effect produced by L-Glu was a result of the stimulation of Qu-type EAA receptors in the striatum (Table 2). However, since K at a medium dose produced only similar motor effects as L-Glu and Qu, and GDEE can displace K from its binding sites in vitro, the involvement of the K-type EAA receptors cannot be ruled out. The ineffectiveness of HA in inhibiting the motor behavior produced by L-Glu indicates that this action of L-Glu was not mediated by DA receptors. The reason for the testing of GABA and Gly on the motor behavior of L-Glu is that both are inhibitory neurotransmitters and in addition Gly is an allosteric regulator of the NMDA receptor-channel complex (11). The effect of glycine on the duration of the motor behavior induced by L-Glu and the lack of effect of GABA indicate that Gly may be involved, but GABA is not, in the L-Glu-produced motor behaviors.

Movement disorders have been shown to be associated with diseases of the basal ganglia (19). Since the nuclei of the basal ganglia receive glutamatergic projections (6), defects in the release or in the presynaptic uptake of Glu are possible causes of some of the movement disorders.

REFERENCES

1. Ben-Ari, Y.; Tremblay, E.; Ottersen, O. P. Injection of kainic acid into the amygdaloid complex of the rat: an electrographic clinical and histological study in relation to the pathology of epilepsy. Neuroscience 5:515–528; 1980.
2. Beninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173–196; 1983.
3. Carter, C. J. Loss of glutamine synthetase activity in the brain in Huntington's disease. Lancet 1:782–783; 1981.
4. Coyle, J. T.; Schwarcs, R. Lesion of striatal neurons with kainic acid provides a model for Huntington's chorea. Nature 263:244–246; 1976.
5. Divac, J.; Fonnum, F.; Storm-Mathisen, J. High-affinity uptake of glutamate in terminals of corticostriatal axons. Nature 266:377–378; 1977.
6. Fagg, E. G.; Foster, A. C. Amino acid neurotransmitters and their pathways in the mammalian central nervous system. Neuroscience 9:701–719; 1983.
7. Fishman, R. H. B.; Feigenbaum, J. J.; Yanai, J.; Klawans, H. L. The relative importance of dopamine and norepinephrine in mediating locomotor activity. Prog. Neurobiol. 20:55–88; 1983.
8. Fonnum, F.; Storm-Mathisen, J.; Divac, J. Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibers in rat brain. Neuroscience 6:863–873; 1981.
9. Foster, A. C.; Fagg, G. E. Acidic amino acid binding sites in mammalian neuronal membranes: Their characteristics and relationship to synaptic receptors. Brain Res. Rev. 7:103–164; 1984.
10. Foster, A. C.; Fagg, G. E.; Mena, E. E.; Cotman, C. W. L-glutamate and L-aspartate bind to separate sites in rat brain synaptic membranes. Brain Res. 229:246–250; 1985.
11. Foster, A. C.; Fagg, G. E. Taking apart NMDA receptors. Nature 329:395–396; 1987.
12. Giorgio, O.; Meek, J. L. γ-Aminobutyric acid turnover in rat striatum: Effect of glutamate and kainic acid. J. Neurochem. 42: 215–220; 1984.
13. Giorguieff, M. F.; Keruel, M. L.; Glowinski, J. Presynaptic effect of L-glutamic acid on the release of dopamine in rat striatal slices. Neurosci. Lett. 6:73–77; 1977.
14. Godukhin, O. V.; Zharikova, A. D.; Novoselov, V. I. The release of labeled L-glutamic acid from rat neostriatum in vivo following stimulation of frontal cortex. Neuroscience 5:2151–2154; 1980.
15. Hassler, R.; Hang, P.; Nitsch, C.; Kim, J. S.; Paik, K. Effect of motor and premotor cortex ablation on concentrations of amino acids, monoamines, and acetylcholine and on the ultrastructure in rat striatum. A confirmation of glutamate as the specific corticostriatal transmitter. J. Neurochem. 38:1087–1098; 1982.
16. Kim, J. S.; Hassler, R.; Hang, P.; Paik, K. S. Effect of frontal cortex ablation on striatal glutamic acid levels in rat. Brain Res. 132: 370–374; 1977.
17. McGeer, E. G.; McGeer, P. L. Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature 263:517–519; 1976.
18. McGeer, P. L.; McGeer, E. G.; Scherer, U.; Singh, K. A glutamatergic corticostriatal path? Brain Res. 128:369–373; 1977.
19. Marsden, C. D. Movement disorders and the basal ganglia. Trends Neurosci. 9:512–515; 1986.
20. Mitchell, P. R.; Dogett, N. S. Modulation of striatal [^{3}H]glutamic acid release by dopaminergic drugs. Life Sci. 26:2073–2081; 1980.
21. Olney, W. J.; De Gubareff, T. Glutamate neurotoxicity and Huntington's Chorea. Nature 271:557–559; 1979.
22. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
23. Roberts, P. J.; Anderson, S. D. Stimulatory effect of L-glutamate and related amino acids on [^{3}H]dopamine release from rat striatum: an in vitro model for glutamate actions. J. Neurochem. 32:1539–1545; 1979.
24. Roberts, P. J.; McBean, G. J.; Sharif, N. A.; Thomas, E. M. Striatal glutamatergic function: modifications following specific lesions. Brain Res. 235:83–91; 1982.
25. Rowlands, G. J.; Roberts, P. J. Activation of dopamine receptors inhibits calcium-dependent glutamate release from cortico-striatal terminals in vitro. Eur. J. Pharmacol. 62:241–242; 1980.
26. Scatton, B.; Lehmann, J. N-Methyl-D-aspartate-type receptors medi-

ate striatal ^{3}H-acetylcholine release evoked by excitatory amino acids. Nature 297:422–424; 1982.
27. Schwarcz, R.; Coyle, J. T. Striatal lesions with kainic acid: Neurochemical characteristics. Brain Res. 127:235–249; 1978.
28. Schwob, J. E.; Fuller, T.; Price, J. L.; Olney, J. W. Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: A histological study. Neuroscience 5: 991–1014; 1980.
29. Spencer, J. H. Antagonism of cortical excitation of striatal neurons by glutamic acid diethyl ester: evidence for glutamic acid as an excitatory transmitter in the rat striatum. Brain Res. 102:91–101; 1976.
30. Streit, P. Selective retrograde labeling indicating the transmitter of neuronal pathways. J. Comp. Neurol. 191:429–463; 1980.
31. Worms, P.; Willigens, M. T.; Lloyd, K. G. The behavioral effects of systemically administered kainic acid: a pharmacological analysis. Life Sci. 29:2215–2225; 1981.