Pharmacology Biochemistry & Behavior, Vol 29, pp 525–528 © Pergamon Press plc, 1988 Printed in the U S A 0091-3057/88 $3 00 + 00

3-Mercaptopropionic Acid Administration Into the Caudate-Putamen of the Rat Provokes Dyskinesia

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Received 19 June 1987

TOTH, E AND A LAJTHA *3-Mercaptopropionic acid administration into the caudate-putamen of the rat provokes dyskinesia* PHARMACOL BIOCHEM BEHAV **29**(3) 525–528, 1988 —The unilateral administration of 3-mercaptopropionic acid (MPA) through an implanted guide cannula into the caudate-putamen produced dyskinesia in the rat Striatal GABA and dopamine were decreased and the dopamine metabolites 3,4-dihydroxyphenylacetic and homovanillic acid were increased on the MPA-injected side at 2–10 min after the onset of dyskinesia The dyskinetic movements were blocked by GABA or α-aminooxaloacetic acid but not by glycine or haloperidol

Striatum GABA Dyskinesia

STUDIES on movement disorders, including drug-induced tardive dyskinesia (TD), indicated that in addition to dopamine (DA) and acetylcholine (ACh) GABA is also involved [5,17] It was proposed that TD may be caused by neuroleptic-induced destruction of GABAergic neurons in the caudate nucleus and putamen The activity of glutamic acid decarboxylase (GAD) was decreased in the substantia nigra of chronically haloperidol-treated rats, which showed chewing movements [7], and also in the postmortem brains of cebus monkeys with neuroleptic-induced dyskinesia [8] A monkey with unilateral dyskinesia showed a decrease in GAD only in the affected side [8] Other GABA-related changes following chronic neuroleptic administration, such as decrease in the turnover rate [11], increase in the binding [6] and rise in the receptor sensitivity of GABA [16], have been shown in the striatum Injection of GABA antagonists (picrotoxin, bicuculline) in the striatum of rats provoked dyskinetic activities [10, 13, 19, 21]

Enhancement of GABA function by inhibiting GABA transaminase in humans with TD had a beneficial effect Gamma-acetylenic-GABA [4] and gamma-vinyl-GABA [9,24] reduced TD Clonazepam, one of the benzodiazepines known to potentiate GABA mechanisms, also reduced TD in humans and monkeys [3] GABA agonists were active against tardive dyskinesia in man [14,22], and decreased the symptoms of oral dyskinesia in monkeys [1]

Since the basal ganglia appear to be involved in the dyskinetic movements and GABA is the major inhibitory transmitter in this region of the brain, we investigated the motor effect of inhibition of GABA synthesis in the striatum, the largest cell mass of the basal ganglia The synthesis of GABA was inhibited in the striatum by focal injection of 3-mercaptopropionic acid (MPA), an inhibitor of GAD in the caudate-putamen through a chronically implanted guide cannula The objective of this study was to establish whether there is a direct relationship between striatal GABA level reduction and dyskinesia The possible involvement of other transmitter systems in the mediation of GABA-related dyskinesia was also studied

METHOD

Stereotaxic Implantation of the Guide Cannula

The surgery was performed under chloral hydrate (350 mg/kg) anesthesia A 26-gauge stainless steel guide cannula targeted vertically 1 mm above the site of injection was implanted unilaterally on the right side according to the coordinates of the Pellegrino rat brain atlas [15] with a Kopf stereotaxic instrument The cannula was fixed with 4 mounting screws (2 mm) and cranioplastic cement (Plastic Products Co , Roanoke, VA) The coordinates with system B were the following *caudate nucleus*, rostral-caudal (RC) 2 4 (2 4 mm anterior to the bregma), medial lateral (ML) 3 0 (3 0 mm lateral to the midline), dorsal ventral (DV) 5 0 (5 0 mm ventral to the surface of the skull), *cerebellum*, RC-8 8, ML 0 0, DV 3 0, *superior colliculus*, RC 5 8, ML 1 5, DV 3 5, *substantia nigra*, RC-3.2, ML 3 0, DV 8 0, *corpus callosum*, RC 1 4, ML 3 0, DV 2 0, *frontal cortex*, RC 5 6, ML 1 5, DV 2 5, *periventricular gray matter*, RC-6 0, ML 0 7, DV 3 5 The incisor bar was set 5 0 mm above the interaural line. After surgery the rats were handled repeatedly and mock-injected during a 7-day recovery to habituate them to the injection procedure

Injection of 3-MPA and Monitoring of Motor Function

Seven days after surgery the rats were injected without

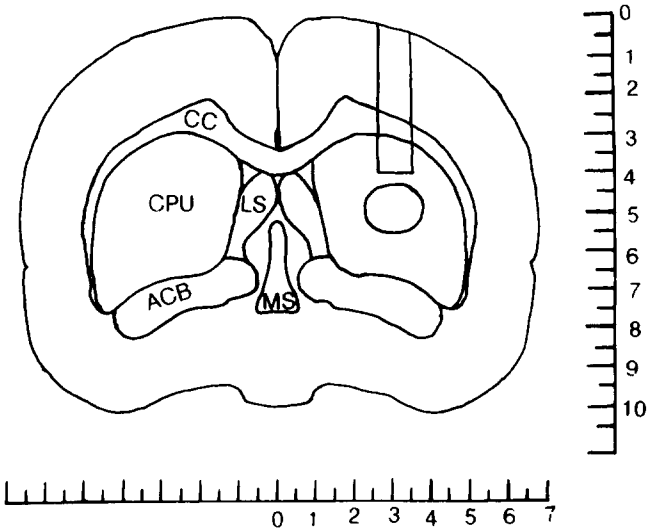

FIG 1 Schmatic reconstruction of the track of guide cannula and the site of injection in the caudate-putamen 2 4 mm anterior to bregma

restriction with 0 3–4 0 μmol of MPA (pH 7 4) in 1 μl at 0 25 μl/min After injection the rats were put in a 50×50×40 cm transparent plastic bin and their motor behavior was observed visually and recorded

Verification of the Site of Injection

To verify the site of injection in the caudate-putamen (Fig 1), the rats were killed with an overdose of chloral hydrate (900 mg/kg) injected IP, then perfused intracardially with isotonic saline followed by 10% formalin Each 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 [15] To estimate the spread of MPA in the striatum 1 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

Determination of GABA, DA, and DA Metabolites

Rats were decapitated with a guillotine in the cold room so that the head fell into a 0 9% (wt /vol) NaCl solution maintained at 0 5°C [27] The heads were moved through the fluid for 5 min to accelerate cooling Then the brain was removed and both striata were dissected out and frozen in dry ice

For the determination of GABA the striatum was homogenized in 1 M perchloric acid The supernatant after centrifugation at 30,000×g for 20 min was analyzed on an amino acid analyzer [25]

The level of DA and DA metabolites was measured with HPLC The striata were homogenized in 0 05 M perchloric acid containing 0.1% cysteine, and the supernatant after centrifugation was injected into a Biophase ODS 5-μm column the electrode set at +800 mV potential [18]

Materials

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

TABLE 1

STRIATAL GABA LEVELS AFTER UNILATERAL INJECTION OF 3-MERCAPTOPROPIONIC ACID IN CAUDATE-PUTAMEN

Experiments	μmol/g GABA Right	Left	*p*	Right/ Left
Control	2 26±0 24	2 41±0 18	NS	0 94
2–10 min after onset of dyskinesia	1 14±0 36	2 33±0 64	<0 01	0 49
3–30 min after dyskinesia ceased	1 54±0 37	2 15±0 54	<0 05	0 72

Rats were injected with 200 μg of MPA in the right caudate-putamen and killed 3–10 min after the onset or 3–30 min after the end of dyskinesia The values are the means±S D of 4–6 experiments

Chemicals 3-Mercaptopropionic acid, GABA, haloperidol, α-aminooxaloacetic acid, Evans blue, and glycine were obtained from Sigma Chemical Co , St Louis, MO

RESULTS

MPA injected in the right caudate-putamen of rats induced periodic involuntary movements of the head, forelimbs, and trunk in 5–10 min Representative pictures of the main dyskinetic movements, which were repeated in every 2–10 min, are shown in Fig 2 All dyskinetic movements were reversible and dose-related The threshold dose, 60 μg, induced head and contralateral forelimb movements The higher doses (100 to 400 μg), in addition, produced bilateral forelimb and contralateral torso movements The onset of dyskinesia was similar after different doses but the duration of dykinesia was longer and affected more areas of the body at larger doses The duration was 25, 50, 70, and 300 min after 60, 100, 200, or 400 μg MPA respectively

The level of GABA in the right striatum was reduced by about 50% in 3–10 min after the onset of dyskinesia, and it was restored to 70% of its normal level in 3–30 min after all dyskinetic movements ceased There was no change in GABA levels in the left striatum (Table 1) Since GABA regulates the DA system in the striatum, we also measured the level of DA and dopamine metabolites in the striatum after MPA injection DA level was also reduced in the right striatum after MPA injection, but the levels of DOPAC and HVA were increased After the end of the dyskinetic movements some of the loss in DA levels was restored but the levels of DOPAC and HVA remained elevated (Table 2)

All dyskinetic movements were blocked by GABA 5 μmol 30 min before, or 2 μmol with the injection of MPA and also by 25 μg/g α-aminooxaloacetic acid a (GABA-T inhibitor) 5 hr before MPA injection Haloperidol and glycine were ineffective The focal injection of 200 μg MPA produced convulsion in cerebellum, contraversive rotation, and convulsion in colliculus superior, hyperactivity and wet shakes in substantia nigra, no effect in corpus callosum, convulsion in frontal cortex and hyperactivity in the periventricular gray matter

DISCUSSION

These results show that a unilateral reduction of striatal

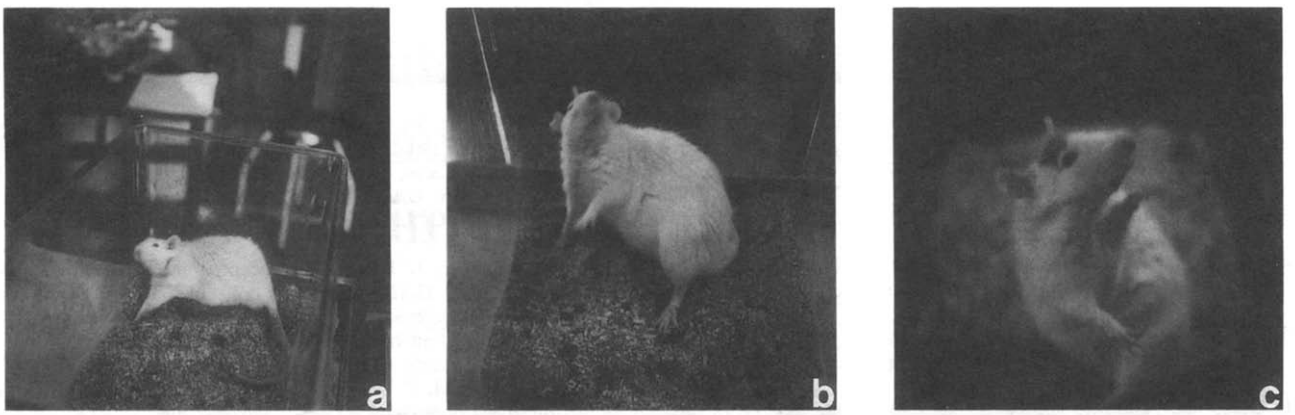

FIG 2 The three main types of dyskinetic movements after the unilateral injection of 60 μg (a), 100 μg (b), or 200–400 μg (c) of MPA in the caudate-putamen

TABLE 2

LEVELS OF DOPAMINE AND ITS METABOLITES IN STRIATUM AFTER INJECTION OF 3-MERCAPTOPROPIONIC ACID IN THE CAUDATE-PUTAMEN

	DOPAC		DA		HVA		DOPA + HVA/DA	
				μg/g				
Experiments	R	L	R	L	R	L	R	L
Control	0 91±0 12	1 10±0 15	10 0 ±0 21	10 1 ±0 28	1 06±0 13	1 29±0 21	0 20±0 08	0 23±0 05
3–10 min after onset of dyskinesia	2 88±1 21*	1 54±0 41	5 38±1 93†	10 3 ±2 74	2 83±0 65‡	1 67±0 47	0 96±0 16	0 37±0 12
3–30 min after dyskinesia ceased	4 01±1 68	1 87±1 13	8 76±2 23	8 69±1 50	3 16±1 09	2 70±0 83	0 80±0 11	0 56±0 32

Rats were injected in the right striatum with 200 μg of MPA and killed 3–10 min after the onset or 3–30 min after the end of the dyskinesia The values are the means±S D of 4–5 experiments R, L, right and left striatum

*$p<0$ 02

†$p<0$ 05

‡$p<0$ 01 compared to left

GABA by MPA to about half of its physiological level in rat results in dyskinesia These dyskinetic movements are reversible, ending as soon as the level of GABA rises up to 70% of its normal level (Table 1) This MPA-induced dyskinesia can be blocked by increasing the striatal GABA level either by prior or simultaneous injection with MPA Prior blockage of the DA receptors either by systemic or focal injection of haloperidol had no inhibitory effect, indicating that the postsynaptic DA receptors are not involved in the mediation of MPA-induced dyskinesia The concomitant decrease of DA and increase of DA metabolites (Table 2) with reduction of GABA in the striatum are probably due to disinhibition of DA release GABA has been shown to exert an inhibitory control on the nigrostriatal DA system [2,26]

It has been hypothesized that the cause of movement disorder is an imbalance between the levels of DA, ACh [12,23] and GABA [20] in the basal ganglia Our results support this theory, but in the case of GABA the imbalance caused by reduction provoked dyskinesia, whereas the imbalance produced by the increase in the striatal GABA level did not. For DA and ACh, both excess and reduced level provoked dyskinetic movements The cause of this difference is probably the overall inhibitory nature of the GABA

Since MPA was not shown to have neurochemical effects in vivo other than the inhibition of GAD, the dyskinesia that it provoked in the striatum was probably due to the reduction of the GABA level This is supported by the finding of correlation between the striatal GABA levels and the onset and end of dyskinesia Since MPA does not seem to cause tissue damage at the doses used and the rats were completely normal even after repeated intracaudate injections, this model may be useful for studying movement disorders

ACKNOWLEDGEMENT

We thank Mrs Audrey Hashim for her excellent help in doing the determination of the levels of catechol derivatives

REFERENCES

1 Barany, S and L M Gunne Pharmacological modification of experimental tardive dyskinesia *Acta Pharmacol Toxicol* **45:** 107–111, 1979

2 Bartholini, G Interaction of striatal dopaminergic cholinergic and GABAergic neurons relation to extrapyramidal function *Trends Pharmacol Sci* **1:** 138–140, 1980

3 Borbruff, A , G Gardos, D Tarsy, R M Rapkin, J O Cole and P Moore Clonazepam and phenobarbital in tardive dyskinesia *Am J Psychiatry* **138:** 189–193, 1981

4 Casey, D E , J Gerlach, G Magelund and T R Christensen γ-Acetylenic GABA in tardive dyskinesia *Arch Gen Psychiatry* **37:** 1376–1379, 1980

5 Fibiger, H C and K G Lloyd Neurobiological substrates of tardive dyskinesia The GABA hypothesis *Trends Neurosci* **7.** 462–464, 1984

6 Gale, K Chronic blockage of dopamine receptors by antischizophrenic drugs enhances GABA binding in substantia nigra *Nature* **283:** 569–570, 1980

7 Gunne, L M and J E Haggstrom Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia *Psychopharmacology (Berlin)* **81.** 191–194, 1983

8 Gunne, L M , J E Haggstrom and B Sjoquist Persistent neuroleptic-induced dyskinesia associated with regional changes within brain GABA and dopamine systems *Nature* **309:** 347–349, 1984

9 Korsgaard, S , D E Casey and J Gerlach Effect of gamma-vinyl GABA in tardive dyskinesia *Psychiatry Res* **8.** 261–269, 1983

10 McKenzie, G M and K Vilk Chemically induced choreiform activity Antagonism by GABA and EEG patterns *Exp Neurol* **46:** 229–243, 1975

11 Mao, C C , D L Cheney, E Marco, A Revuelta and E Costa Turnover times of gamma aminobutyric acid and acetylcholine in nucleus caudatus, nucleus accumbens, globus pallidus, and substantia nigra Effects of repeated administration of haloperidol *Brain Res* **132:** 375–379, 1977

12 Marsden, C D Basal ganglia disease *Lancet* **2:** 21–27, 1982

13 Marsden, C D , B S Meldrum, C Pycock and D Tarsy Focal myoclonus produced by injection of picrotoxin into the caudate nucleus of the rat *J Physiol* **246:** 96P, 1974

14 Morselli, P L , V Fournier, L Bossi and B Musch Clinical activity of GABA agonists in neuroleptic and L-dopa-induced dyskinesia In *Dyskinesia Research and Treatment,* edited by D E Casey, T Chase, A V Christensen and J Gerlach Berlin Springer-Verlag, 1985, pp 128–136

15 Pellegrino, L J , A S Pellegrino and A J Cushman *A Stereotaxic Atlas of the Rat Brain* New York Plenum Press, 1975

16 Scheel-Kruger, J , G Magelund and M C Olianas Role of GABA in the striatal output system globus pallidus, nucleus entopenduncularis, substantia nigra, and nucleus subthalamicus In *GABA and the Basal Ganglia,* edited by G DiChiara and G L Gessa New York Raven Press, 1981, pp 165–186

17 Scheel-Kruger, J The GABA receptor and animal behaviour evidence that GABA transmits and mediates dopaminergic functions in the basal ganglia and the limbic system In *GABA Receptors,* edited by S J Enna Clifton, NJ Humana Press, 1983, pp 215–255

18 Sershen, H , M F Mason, A Hashim and A Lajtha Effect of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on age-related changes in dopamine turnover and transporter function in the mouse striatum *Eur J Pharmacol* **113.** 135–136, 1985

19 Slater, P and L S Dickinson Role of acetylcholine and dopamine in myoclonus induced by intrastriatal picrotoxin *Neurosci Lett* **28:** 253–257, 1982

20 Stahl, S M Neuropharmacology of movement disorders In *Movement Disorder,* edited by N S Shah and A Donald New York Plenum Publishing, 1986, pp 1–36

21 Standefer, J M and E R Dill The role of GABA in dyskinesia induced by chemical stimulation of the striatum *Life Sci* **21·** 1515–1520, 1977

22 Tamminga, C A , J W Crayton and T N Chase Improvement in tardive dyskinesia after muscimol therapy *Arch Gen Psychiatry* **36:** 595–598, 1979

23 Tarsy, D Dopamine-acetylcholine interaction in the basal ganglia In *Basic and Clinical Aspects of Neurotransmitter Function,* edited by W S Fields Basel S Karger, 1976, pp 1–15

24 Tell, G P , P J Schechter and J Koch-Weser Effects of gamma-vinyl GABA *N Engl J Med* **305.** 581–582, 1981

25 Toth, E and A Lajtha Effect of protein-free diet on the uptake of amino acids by the brain in vivo *Exp Neurol* **68·** 443–452, 1980

26 Wood, P L Action of GABAergic agents on dopamine metabolism in the nigrostriatal pathway of the rat *J Pharmacol Exp Ther* **222:** 674–679, 1981

27 Wood, J D , E Kurylo and J D Newstead Aminooxaloacetic acid induced changes in γ-aminobutyric acid metabolism at the subcellular level *Can J Biochem* **56:** 667–672, 1978