Pharmacology Biochemistry & Behavior, Vol. 16, pp. 7–11, 1982. Printed in the U.S.A.

Possible Mechanism of 5-Methoxy-N,N-Dimethyltryptamine-Induced Turning Behaviour in DRN Lesioned Rats

T. P. BLACKBURN,[1] B. COX, C. G. HEAPY AND T. F. LEE

Bioscience Department II, ICI Pharmaceuticals Division
Alderley Park, Macclesfield, Cheshire, SK10 4TG, U. K.

Received 3 June 1981

BLACKBURN, T. P., B. COX, C. G. HEAPY AND T. F. LEE. *Possible mechanism of 5-methoxy-N,N-dimethyltryptamine-induced turning behaviour in DRN lesioned rats.* PHARMAC. BIOCHEM. BEHAV. 16(1) 7–11, 1982.—5-Methoxy-N,N-dimethyltryptamine (5-MeODMT) (7.5 mg/kg SC) caused a contralateral turning in rats with a unilateral lesion of the dorsal raphe nucleus (DRN). This turning behaviour was blocked by pretreatment with putative 5-HT antagonists, methysergide, cyproheptadine and cinanserin. The peripheral 5-HT antagonist, xylamidine, also prevented the response to 5-MeODMT. Of the other neurotransmitter antagonists, only haloperidol was active, hyoscine, picrotoxin, naloxone and strychnine were ineffective. Pretreatment with α-methyl-p-tyrosine (α-MT) also significantly reduced the turning response to 5-MeODMT. These results indicate that a central dopaminergic system is involved in 5-MeODMT-induced turning behaviour. This suggestion is supported by the finding that an ipsilateral turning in response to 5-MeODMT was observed in the rats with additional 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle (MFB). The possible mechanisms by which 5-MeODMT induced turning in DRN lesioned rats are discussed.

Dorsal raphe lesion Turning behaviour 5-MeODMT 5-HT receptor Dopaminergic system

TURNING behaviour in rats is commonly observed after unilateral brain lesions induced by either neurotoxin injection or by electrothermal or radiofrequency damage. It can also be induced by direct injection of drugs into the basal ganglia (for reviews see [12,24]). A widely accepted explanation of this turning behaviour is that the lesion causes a functional imbalance in the dopaminergic systems of the two sides of the brain.

Since Ungerstedt first proposed this hypothesis [31], much work has been carried out in an attempt to characterise the response and a variety of other neurotransmitters have been claimed to be involved [12,24]. We have previously reported that 5-HT can induce a turning response after a unilateral 5,7-dihydroxytryptamine (5,7-DHT) lesion of the dorsal raphe nucleus (DRN)of the rat [2]. Previous studies [13, 16, 29] have also reported rotational behaviour after lesions of raphe cell bodies or axons using different lesioning techniques. In our present study direct 5-HT agonists caused a contralateral turning, whereas indirectly acting 5-HT agonists caused an ipsilateral turning [2]. The contralateral turning may be explained by the existence of so-called supersensitive receptors in the substantia nigra of the lesioned side [1]. However, the precise functional role of 5-HT in this region has not yet been finally established, although an electrophysiological study has shown that nigral neurones can be inhibited by iontophoretic application of 5-HT [9]. Usually the turning behaviour, whatever the initiator, is finally mediated via a dopaminergic system. However, recently the turning behaviour induced by direct injection of GABAergic drugs into the substantia nigra has produced conflicting reports as to the involvement of dopamine [16, 17, 18, 28].

Therefore, the aim of the present study was to determine whether the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), induced turning depends on a central dopaminergic system. It was also decided to investigate the possibility that other neurotransmitters were involved since a number of compounds can induce turning response after intranigral injection [23].

METHOD

Surgical Procedures

Adult male Alderley Park SPF strain rats (180–200 g) were used. The rats were anaesthetised with halothane (3% v/v O_2) and a unilateral lesion of the DRN was made by means of a stereotaxic injection of 5,7-DHT (16 μg salt/2 μl 0.2% ascorbic acid solution injected one hour after an IP injection of pargyline 50 mg/kg) using a David Kopf stereotaxic frame as described previously [2]. In some experiments a second lesion was carried out in the rats by injecting 6-hydroxydopamine (6-OHDA) (8 μg/2 μl 0.2% ascorbic acid injected 30 min after an IP injection of desipramine 25 mg/kg) into the medial forebrain bundle (MFB) ipsilateral to the

[1]All correspondence should be addressed to T. P. Blackburn.

TABLE 1

EFFECTS OF 5-MeODMT ON THE TURNING BEHAVIOUR OF DRN LESIONED RATS†

Drugs	Dose (mg/kg SC)	Mean total no. of turns*/2 hr ±S.E.	No. of animals tested
Saline		−11 ± 12	6
5-MeODMT	1.0	+89 ± 25†	8
5-MeODMT	2.5	+122 ± 51‡	9
5-MeODMT	7.5	+231 ± 45§	13
5-MeODMT	10.0	+273 ± 67‡	24

*+ indicates contralateral turning and − indicates ipsilateral turning with respect to the lesion side.

Significant difference from saline controls, †$p<0.05$, ‡$p<0.01$; §$p<0.005$.

5,7-DHT lesion (co-ordinates: AP=1.4 mm posterior to bregma, L=1.7 mm, D=7.5 mm from dura, according to the atlas of Pellegrino and Cushman [23]). Sham-operated control rats were prepared using the same procedure except that 0.2% ascorbic acid replaced the neurotoxin injection. After completion of an experiment, histological verification of lesion site and biochemical estimations of the selectivity of neurotoxin were carried out as previously described [2].

Behavioural Procedures

On the fourth day after the DRN lesion all rats were assessed for turning behaviour by challenging with 5-MeODMT (7.5 mg/kg SC). The turning behaviour was assessed in automated rotometers [3] and recorded at 10 min intervals after drug injection for a period of two hours. The lesion was considered successful (approximately 75% of animals lesioned) when a rat made at least 200 turns within the 2 hr assessment period. Drug-induced turning behaviour was then assessed with at least 72 hr intervals between successive drug injections for a period of up to 2 months. Sham-operated or control rats were tested with the same procedure. Comparisons between simultaneously tested groups were made using the non-parametric Wilcoxon Signed Rank test.

Drug Used

Cinanserin oxalate (obtained as a gift from Dr. R. J. Pearce, ICI), cyproheptadine hydrochloride (Merck), desipramine hydrochloride (Geigy Pharmaceuticals), 5,7-DHT creatinine sulphate (Sigma), haloperidol (Serenace, Searl & Co., Ltd.), 6-OHDA hydrobromide (Sigma), hyoscine hydrobromide (BDH), 5-MeODMT (Sigma), D,L-α-methyltyrosine (α-MT) methyl ester hydrochloride (Sigma), methysergide bimaleate (Sandoz), naloxone hydrochloride (Endo. Lab., Inc.), pargyline hydrochloride (Sigma), picrotoxin (Sigma) and strychnine sulphate (Macfarlane Smith). Drugs were injected IP in a dose volume of 1 ml/kg except 5-MeODMT which was given SC. All doses refer to the free base unless otherwise stated.

RESULTS

Effect of Serotonergic Antagonists on 5-MeODMT-Induced Turning

Subcutaneous injection of 5-MeODMT into DRN-lesioned rats caused a dose related contralateral turning behaviour (Table 1). A submaximal dose of 5-MeODMT (7.5 mg/kg) was then selected since the aim of this study was to examine the effects of various antagonist drugs at doses reported to inhibit drug-induced rotational behaviour. Intraperitoneal pretreatment with the 5-HT antagonist, methysergide 10 mg/kg, cyproheptadine 5 mg/kg and cinanserin 10 mg/kg [20,29], significantly reduced the contralateral turning induced by 5-MeODMT (Table 2). A three hour pretreatment with the peripheral 5-HT antagonist, xylamidine 1 mg/kg [5], also reduced the turning response to

TABLE 2

EFFECTS OF 5-HT ANTAGONISTS ON 5-MeODMT-INDUCED TURNING IN DRN LESIONED RATS

Drugs	Dose (mg/kg SC)	Pretreatment time (hr)	Mean total no. of turns/2 hr ±S.E.	No. of animals tested
5-MeODMT	7.5		236 ± 50	8
5-MeODMT + methysergide	7.5 10.0	1	129 ± 33†	8
5-MeODMT	7.5		233 ± 21	8
5-MeODMT + cyproheptadine	7.5 5.0	1	107 ± 20*	7
5-MeODMT	7.5		255 ± 26	8
5-MeODMT + cinanserin	7.5 10.0	1	137 ± 31*	8
5-MeODMT	7.5		230 ± 36	4
5-MeODMT + xylamidine	7.5 1.0	3	114 ± 23*	4

Significant difference from appropriate controls, *$p<0.01$; †$p<0.025$.
Antagonists given intraperitoneally.

TABLE 3

EFFECTS OF OTHER NEUROTRANSMITTER ANTAGONISTS ON 5-MeODMT-INDUCED TURNING IN DRN LESIONED RAT

Drugs	Dose (mg/kg SC)	Pretreatment time (min)	Mean ± S.E. total no. of turns/2 hr	No. of animals tested
5-MeODMT	7.5	60	199 ± 26	5
5-MeODMT + haloperidol	7.5 0.3		3 ± 2*	5
5-MeODMT	7.5	15	217 ± 18	7
5-MeODMT + naloxone	7.5 2.0		236 ± 28	7
5-MeODMT	7.5	30	234 ± 31	5
5-MeODMT + hyoscine	7.5 2.5		225 ± 35	5
5-MeODMT	7.5	30	233 ± 33	5
5-MeODMT + picrotoxin	7.5 2.5		246 ± 25	5
5-MeODMT	7.5	10	221 ± 26	4
5-MeODMT + strychnine	7.5 0.5		226 ± 30	4

Significant difference from appropriate controls, *$p<0.005$.
Antagonists given intraperitoneally.

5-MeODMT (Table 2). None of the antagonists caused any significant turning behaviour on their own.

Effects of Other Neurotransmitter Antagonists on 5-MeODMT-induced turning

An hour pretreatment with haloperidol 0.3 mg/kg [20,29] completely abolished the turning response to 5-MeODMT (Table 3). In contrast, intraperitoneal pretreatment with either hyoscine 2.5 mg/kg, naloxone 2 mg/kg, picrotoxin 2.5 mg/kg or strychnine 0.5 mg/kg had no effect on 5-MeODMT-induced turning in DRN lesioned rats at the subconvlusive doses tested (Table 3). No apparent changes in behaviour occurred after pretreatment with these antagonists and the doses used did not induce catalepsy.

Effect of α-MT on 5-MeODMT-Induced Turning

The turning response to 5-MeODMT was significantly reduced after 8 hr pretreatment with α-MT (250 mg/kg IP) (control: 246±23; α-MT pretreated: 71±27, n=4; $p<0.005$). No obvious behavioural change was observed after α-MT pretreatment.

Effects of MFB Lesion on 5-MeODMT-Induced Turning

Rats which responded to 5-MeODMT (at least 200 turns in 2 hrs) received a second lesion by injection of 6-OHDA into the MFB. The animals were tested 14 days after surgery and the results are shown in Table 4. Rats with a DRN lesion gave the usual contralateral turning response to 5-MeODMT. A group of rats which had only a MFB lesion did not exhibit any significant turning behaviour when injected with 5-MeODMT. However, when the DRN lesioned rats received a second MFB lesion the usual contralateral turning response was converted into a significant ipsilateral turning response.

TABLE 4

EFFECTS OF DIFFERENT BRAIN LESIONS ON 5-MeODMT (7.5 mg/kg SC)-INDUCED TURNING IN RATS

Site of lesion	Drugs	Mean total no. of turns*/2 hr ±S.E.	No. of animals tested
DRN	saline	− 14 ± 12	7
	5-MeODMT	+238 ± 13‡	4
MFB	saline	+ 11 ± 6	5
	5-MeODMT	− 38 ± 17	7
DRN+MFB	saline	+ 11 ± 14	6
	5-MeODMT	−188 ± 60†	4

*+ indicates contralateral turning and − indicates ipsilateral turning with respect to the lesions side.
Significant difference from appropriate saline controls, †$p<0.05$, ‡$p<0.01$.

DISCUSSION

We have previously reported that an apparent supersensitivity develops to 5-HT agonists after DRN lesions and that the receptors involved are located within the zona reticulata of the SN [1]. Activation of these receptors is known to result in a contralateral turning behaviour, but the precise mechanism by which the turning is produced is unclear. It was decided therefore to determine whether other neurotransmitter systems were involved in this response, as recent reports suggest a wide variety of neurotransmitters to be present in the SN [7,27]. Antagonist studies with 5-HT antagonists methysergide, cyproheptadine and cinanserin confirm previous reports that they block 5-MeODMT induced turning [20,29], indicating that 5-MeODMT was indeed

acting via 5-HT receptors. However, turning response could also be reduced by the peripheral 5-HT antagonist xylamidine [5], therefore the location of the antagonism needs to be reconsidered. Either a peripheral 5-HT system is involved in the response or xylamidine reaches some central sites (e.g., 5-HT spinal sites) which are believed to mediate the behavioural response [15]. Experiments are underway in an attempt to resolve this problem.

This study has also confirmed that haloperidol can antagonise 5-MeODMT-induce turning [20,29], suggesting that a dopaminergic system is involved after activation of 5-HT receptors. This view is stregthened by the finding that the turning was prevented by depletion of dopamine with α-MT and that an MFB lesion, which would deplete forebrain dopamine, changes the contralateral turning into an ipsilateral turning. Since an MFB lesion on its own did not produce an animal which turned in response to 5-MeODMT, then it is unlikely that 5-MeODMT acts directly on dopaminergic systems. It is most likely that dopamine neurones represent the final common pathway.

If the site of action of 5-MeODMT is within the SN then the contralateral turning could be explained in the following way. It is known that 5-HT neurones form axo-dendritic synapses in the SN [22]. Thus a unilateral lesion increases the number of receptors on the dendrites in the SN on the same side as the lesion (i.e., denervation supersensitivity). Injection of 5-MeODMT acts preferentially on the supersensitive dendritic receptors. Since a contralateral turning occurs and dopamine neurones are involved then this suggests increased activity on the side of lesion. However, bearing in mind that 5.HT is reported to be inhibitory in the SN [9], the only reasonable explanation is that 5-MeODMT prevents DA output from the dendrites to reduce its own autoinhibitory actions. Thus dopaminergic activity at the nigrostriatal terminals in the striatum increases and contralateral turning results. There is some support for this idea since others have reported an increase in nigral DA turnover after lesions of 5-HT tracts [8, 13, 20]. This hypothesis does not apparently fit with the suggestion that 5-HT can release DA from cat SN *in vivo* [4].

An alternative explanation is that 5-HT acts to inhibit some as yet undefined nigral inhibitory system and the resulting disinhibition allows contralateral turning. There are a number of possible candidates for this role. Thus the striatonigral GABA pathway is believed to be inhibitory [7] but since picrotoxin, in a dose reported to antagonise the turning responses to central injections of GABA-mimetics [19], failed to antagonise 5-MeODMT, then a 5-HT-GABA interaction seems unlikely. Indeed since destruction of 5-HT fibres results in a reduced nigral [^{3}H]-GABA binding [10] and since GABA can inhibit nigral 5-HT release [26], then the connection may be GABA to 5-HT not the reverse.

Opiate receptors have also been shown to exist on the nigro-striatal neurones and activation of these receptors influences nigrostriatal activity [11,14]. Therefore endogenous opioids may also be candidates for the putative inter-neurone transmitter. However, since naloxone, in a dose reported to antagonise morphine-induced turning [25], failed to prevent 5-MeODMT-induced turning then an endogenous opioid link also seems unlikely.

Cholinergic and glycinergic neurones are also claimed to be present within the SN [7] but since neither hyoscine nor strychnine modified 5-MeODMT-induced turning then these two interneurones also do not appear to be involved.

Therefore from this study the most reasonable explanation for the effects of 5-MeODMT is that it is acting on 5-HT receptors located on dendrites of DA neurones in the SN as the response to both agonists is similar following SN administration (unpublished data). Although, possible intermediate neurotransmitters (e.g., Substance P, TRH, etc.) still require investigation. These investigations will however rely on the discovery of suitably selective antagonists.

REFERENCES

1. Blackburn, T. P., B. Cox, C. G. Heapy, T. F. Lee and D. N. Middlemiss. Supersensitivity on nigral serotonin receptors and rat rotational behaviour. *Eur. J. Pharmac.* **71:** 343–346, 1981.
2. Blackburn, T. P., G. A. Foster, C. G. Heapy and J. D. Kemp. Unilateral 5,7-dihydroxytryptamine lesions of the dorsal raphe nucleus (DRN) and rat rotational behaviour. *Eur. J. Pharmac.* **67:** 427–438, 1980.
3. Blackburn, T. P., A. B Shore and A. McNab. A microcomputer based monitoring system for rat rotational behaviour. *Br. J. Pharmac.* **69:** 347, 1980.
4. Cheramy, A., V. Leviel and J. Glowinski. Dendritic release of dopamine in the substantia nigra. *Nature* **289:** 537–542, 1981.
5. Copp, F. C., A. F. Green, H. F. Hadson, A. W. Randall and M. F. Sim. New peripheral antagonist of 5-hydroxytryptamine. *Nature* **214:** 200–201, 1967.
6. Creese, I., D. R. Burt and S. H. Snyder. The dopamine receptor: Differential binding of d-LSD and related agents to agonist and antagonist states. *Life Sci.* **17:** 1715–1720, 1975.
7. Dray, A. The striatum and substantia nigra: A commentary on their relationships. *Neuroscience* **4:** 1407–1439, 1979.
8. Dray, A., J. Davis, N. R. Oakley, P. Tongroach and S. Vellucci. The dorsal and medial raphe projections to the substantia nigra in the rat: Electrophysiological, biochemical and behavioural observations. *Brain Res.* **151:** 431–442, 1978.
9. Dray, A., T. J. Goyne, N. R. Oakley and T. Tanner. Evidence for the existence of a raphe projection to the substantia nigra in rat. *Brain Res.* **113:** 45–57, 1976.
10. Gale, K. GABA receptors in rat substantia nigra: Changes in response to lesions and chronic drug treatment. *Soc. Neurosci. Abstr.* **9:** 71, 1979.
11. Gale, K., F. Moroni, K. Kumakura and A. Guidotti. Opiate-receptors in substantia nigra: Role in the regulation of striatal tyrosine hydroxylase activity. *Neuropharmacology* **18:** 427–430, 1979.
12. Giambalvo, C. T. and S. R. Snodgrass. Biochemical and behavioural effects of serotonin neurotoxins on the nigrostriatal dopamine system: Comparison of injection sites. *Brain Res.* **152:** 555, 1978.
13. Giambalvo, C. T. and S. R. Snodgrass. Effect of p-chloramphetamine and 5,7-dihydroxytryptamine on rotation and dopamine turnover. *Brain Res.* **149:** 453–467, 1978.
14. Glick, S. D., T. P. Jerussi and L. N. Fleischer. Turning in circles: The neuropharmacology of rotation. *Life Sci.* **18:** 889–896, 1976.
15. Iwamoto, E. T. and E. L. Way. Circling behaviour and stereotypy induced by intranigral opiate microinjections. *J. Pharmac. exp. Ther.* **203:** 347–359, 1977.
16. Jacobs, B. L. and H. Klemfuss. Brain stem and spinal cord mediation of serotoninergic behavioural syndrome. *Brain Res.* **100:** 450–457, 1975.
17. Jacobs, B. L., S. M. Simon, D. D. Riumy and M. E. Trulson. A quantitative model for studying serotonergic function in the rat. *Brain Res.* **124:** 271, 1977.

18. Kaakkola, S. and I. Kaariainen. Circling behaviour induced by intranigral injection of GABA and muscimol in rats. *Psychopharmacology* **68:** 31–36, 1980.
19. Kilpatrick, I. C. and M. S. Starr. Involvement of dopamine in circling responses to muscimol depends on intranigral site of injection. *Eur. J. Pharmac.* **69:** 407–419, 1981.
20. Kozlowski, M. R. and J. R. Marshall. Rotation induced by intranigral injections of GABA agonists and antagonists: Zone specific effects. *Pharmac. Biochem. Behav.* **13:** 561–567, 1980.
21. Martin, G. E., N. L. Papp and C. B. Bacino. Contralateral turning evoked by intranigral microinjection of muscimol and other GABA agonists. *Brain Res.* **155:** 297–312, 1978.
22. Nicolaou, N. M., M. Garcia-Munoz, G. W. Arbuthnott and D. Eccleston. Interactions between serotonergic and dopaminergic systems in rat brain demonstrated by small unilateral lesions of the raphe nuclei. *Eur. J. Pharmac.* **57:** 295–305, 1979.
23. Oppizzi, G., G. Verde, L. Stefano, R. Cozzi, L. Botalla, A. Liazzi and P. G. Chiodini. Evidence for a dopaminergic activity of methysergide in humans. *Clin. Endocr.* **7:** 267–272, 1975.
24. Parizek, J., R. Hassler and I. J. Bak. Light and electron microscopic autoradiography of substantia nigra of rat after intraventricular administration of tritum labelled noepinephrine, dopamine, serotonin and the precursors. *Z. Zellforsch.* **115:** 137–148, 1971.
25. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*, 1st ed. New York: Meredith, 1967.
26. Pycock, C. J. Turning behaviour in animals. *Neuroscience* **5:** 461–514, 1980.
27. Slater, P., C. Blundell and A. R. Crossman. The effects of narcotic analgesics on the turning behaviour of rats with 6-hydroxydopamine-induced unilateral nigrostriatal lesions. *Neuropharmacology* **19:** 187–193, 1980.
28. Soubrie, P., J. L. Montastruc, S. Bourgoin, T. Reisine, F. Artraud and J. Glowinski. *In vivo* evidence for GABAergic control serotonin release in the cat substantia nigra. *Eur. J. Pharmac.* **69:** 483–488, 1981.
29. Straughan, D. W. and T. A. James. Microphysiological and pharmacological studies on transmitters in the substantia nigra. In: *Advances in Pharmacology and Therapeutics*, vol. 2, edited by P. Simon. Oxford: Pergamon Press, 1978, pp. 87–96.
30. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine system. *Acta physiol. scand.* Suppl. **367:** 69–93, 1971.
31. Waddington, J. L. GABAergic mechanism in the substantia nigra. *Nature* **283:** 696–697, 1980.
32. Waddington, J. L. and T. J. Crow. Rotational responses to serotonergic and dopaminergic agonists after unilateral dihydroxytryptamine lesions of the medial forebrain bundle: Co-operative interactions of serotonin and dopamine in neostriatum. *Life Sci.* **25:** 1307–1314, 1979.