Pharmacology Biochemistry & Behavior, Vol. 12, pp. 979–982. Printed in the U.S.A.

Effect of Some β-Carbolines on Phenylethylamine and Apomorphine Stereotypies in Rats

ILKKA KARI,[1] SEPPO RAPAKKO AND MAUNO M. AIRAKSINEN

Department of Pharmacology, University of Kuopio, Kuopio, Finland

Received 4 March 1980

KARI, I., S. RAPAKKO AND M. M. AIRAKSINEN. *Effect of some β-carbolines on phenylethylamine and apomorphine stereotypies in rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 979–982, 1980.—Tetrahydro-β-carbolines (THBC:s) have recently been shown to occur in the human body and their role in mental diseases has been discussed. The effect of THBC:s and some other β-carbolines (25 mg/kg IP) were studied on the stereotypies caused by apomorphine (APO, 2 mg/kg IP) and phenylethylamine (PEA, 50 mg/kg IP) in rats. These effects of dopaminergic drugs like apomorphine as well as of phenylethylamine have sometimes been used as animal models of paranoid schizophrenia. Dose effect relationships were studied from the most potent substances. All β-carbolines studied significantly inhibited APO stereotypy. 6-Methoxyharmalan was most effective followed by β-carboline (BC), tetrahydro-β-carboline (THBC), 1-methyl-THBC, 6-methoxy-THBC and 6-hydroxy-THBC. 6-Methoxyharmalan, 6-hydroxy-THBC and BC inhibited also PEA stereotypy. Other substances studied were ineffective. β-Carbolines did not inhibit PEA and APO stereotypies in the same way. Thus the mode of PEA and APO stereotypies seems to differ, and β-carbolines seem influence these stereotypies by more than one mechanism. If dopamine hypothesis is valid, the β-carbolines formed in human body may protect rather than worsen in paranoid psychoses.

Apomorphine β-Carbolines Tryptolines Phenylethylamine Paranoid psychose Schizophrenia Rat Stereotyped behaviour

THE dopamine hypothesis is perhaps the most accepted biochemical explanation of schizophrenic symptoms, and stereotypies caused by dopaminergic drugs like amphetamine and apomorphine have been used as animal models of paranoid schizophrenia. Borison *et al.* [7] have claimed that the stereotypy caused by β-phenylethylamine in rats may be the best model presently available. Phenylethylamine is an endogenous substance in mammals [6, 13, 19, 35] and highly concentrated in the limbic system [8]. It may be a neuromodulator at adrenergic synapses [28] and a role in paranoid schizophrenia has been suggested [23, 30, 36].

Some tetrahydro-β-carbolines (tryptolines) related to hallucinogenic Harmala alkaloids are normal constituents in the human blood and urine [18, 20, 27] and we have shown mass spectrometrically the occurrence of 1-methyl-1,2,3,4-tetrahydro-β-carboline (methtryptoline, tetrahydroharmane) in human platelets and plasma after alcohol drinking (unpublished observations). The corresponding aromatized β-carboline, harman has also been reported to occur in human platelets [5]. Their main pharmacological actions seem to base on the inhibition of 5-hydroxytryptamine (5HT) uptake [21,24] and the increase of 5HT concentration in brain [2,17]. In higher concentrations β-carbolines also inhibit dopamine and noradrenaline uptake in vitro [3, 21, 26, 32] and they are inhibitors of monoamineoxidase A [9]. Inhibition of some effects of apomorphine by 1,2,3,4-tetrahydro-β-carboline (THBC) [25] and of the toxicity of amphetamine by 6-methoxy-THBC [1] suggest a mild antidopaminergic effect.

We have compared the effect of some β-carbolines on the stereotyped behaviour caused by phenylethylamine and apomorphine.

METHOD

Animals

Male Wistar (Wistar/Af/Han/Mol/Kuo) rats weighing 200–300 g housed in plastic cages (60×26×43 cm) with wire mesh floor for bitings caused by apomorphine. The rats were maintained 14 hours under lighting and 10 hours in darkness and were supplied with normal food and water.

Procedure

β-Carbolines (25 mg/kg IP, in the preliminary test), apomorphine (APO) (2 mg/kg IP) and β-phenylethylamine (PEA, phenetylamine) (50 mg/kg IP) were used. β-Carbolines were injected together with the stereotypogenic drugs. The dose effect relationship was studied from the most potent β-carbolines. The quantitative evaluation of apomorphine stereotypy was based on the number of biting per 30 sec 20,

[1]Present address: Department of Pharmacy, University of Kuopio, P. O. Box 138, SF-70101 Kuopio 10, Finland.

TABLE 1

EFFECT OF β-CARBOLINES ON THE STEREOTYPED BITINGS CAUSED BY APOMORPHINE (APO)

Drugs	Bitings/30 sec (Mean ± SEM)		
	20min	30 min	40 min
Control (APO only)	19.9 ± 0.5	20.0 ± 0.6	21.3 ± 0.6
APO + β-Carboline	1.3 ± 1.3‡	1.7 ± 1.7‡	4.2 ± 2.7‡
APO + 6-methoxyharmalan	0.3 ± 0.3‡	0 ± 0 ‡	0.3 ± 0.3‡
APO + 6-methoxy-THBC§	8.3 ± 3.8†	10.2 ± 4.5†	9.5 ± 3.2†
APO + 6-hydroxy-THBC	12.7 ± 2.6†	11.0 ± 2.6†	10.7 ± 3.1†
APO + 1-Methyl-THBC	5.5 ± 2.6†	9.0 ± 3.9†	7.0 ± 2.9†
APO +THBC	6.5 ± 4.0†	3.0 ± 1.4‡	2.2 ± 1.8‡

In control group 20 rats, in each test groups 6 rats.
*$p<0.05$, †$p<0.01$ and ‡$p<0.001$.
§THBC tetrahydro-β-carboline.

TABLE 2

EFFECT OF β-CARBOLINES ON THE MAXIMAL INTENSITY OF THE STEREOTYPED BEHAVIOUR CAUSED BY PHENYLETHYLAMINE (PEA)

Drugs	Score of stereotypy (Mean ± SEM)
Control (PEA only)	2.6 ± 0.2
PEA + β-carboline	1.6 ± 0.5*
PEA + 6-methoxyharmalan	0.8 ± 0.4‡
PEA + 6-hydroxy-THBC	1.4 ± 0.3†
PEA + 6-methoxy-THBC	2.4 ± 0.1
PEA + 1-methyl-THBC	2.3 ± 0.1
PEA + THBC	2.1 ± 0.1

In control group 20 rats, in test groups 7–10 rats.
*$p<0.05$, †$p<0.01$ and ‡$p<0.001$.

30 and 40 min after the drug administration. Phenetylamine stereotypy developed slowly after several times repeated IP injections and it was evaluated by using the special scale (0–4 points) of Borison *et al.* [7]. The effects of β-carbolines were studied after 1–2 weeks from beginning of daily phenetylamine administration.

Drugs

β-Phenylethylamine chloride (Sigma), apomorphine chloride (Ph.Nord), norharman (β-carboline) chloride (Sigma), 1,2,3,4-tetrahydro-β-carboline (tryptoline, Sigma), 1-methyl-tetrahydro-β-carboline (methtryptoline, Sigma) and 6-methoxyharmalan (6-methoxy-1-methyl-3,4-dihydro-β-carboline, Sigma). 6-Methoxy-1,2,3,4-tetrahydro-β-carboline and 6-hydroxy-1,2,3,4-tetrahydro-β-carboline were synthetized in the Department of Pharmaceutical Chemistry, University of Kuopio [15,16].

Statistical Analysis

The Student's *t*-test was used when comparing two means.

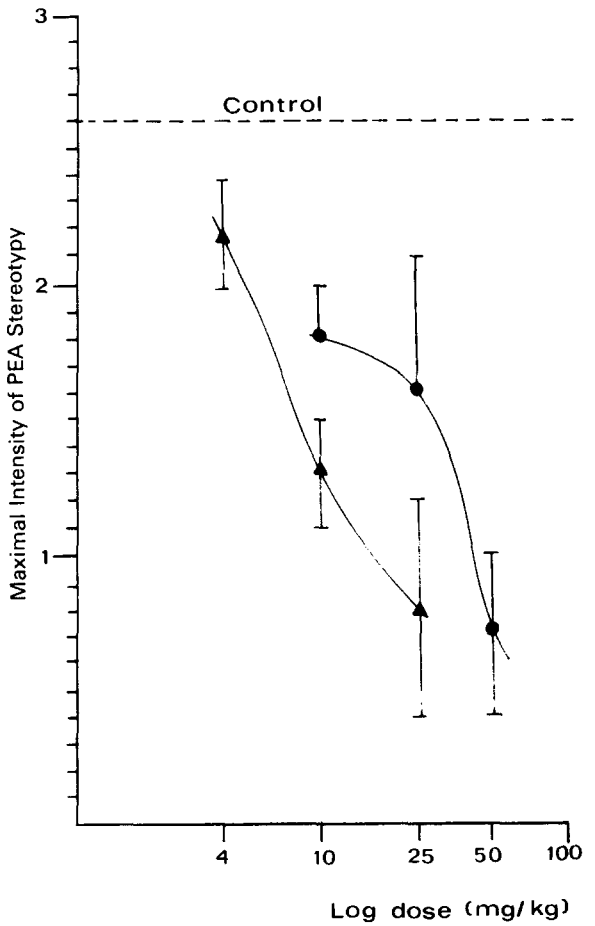

FIG. 1. Dose effect relationship of 6-methoxyharmalan (▲) and β-carboline (●) on the score of PEA stereotypy (Mean ± SEM). Doses of 6-methoxyharmalan were 4 mg/kg (score 2.2 ± 0.2), 10 mg/kg (1.3 ± 0.2***), 25 mg/kg (0.8 ± 0.4***) and of β-carboline 10 mg/kg (1.8 ± 0.2*), 25 mg/kg (1.6 ± 0.2*) and 50 mg/kg (0.7 ± 0.3***). The scores of controls was 2.6 ± 0.1.Control group 30 rats, test groups 6–8 rats. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

RESULTS

All β-carbolines studied inhibited the stereotyped behavior caused by apomorphine (Table 1). 6-Methoxyharmalan was the most effective, followed by β-carboline (norharman) and tetrahydro-β-carboline. 1-Methyl-, 6-methoxy- and 6-hydroxy-derivatives of tetrahydro-β-carboline were slightly weaker, but all these substances had a significant effect. A dose effect relationship was studied for 6-methoxyharmalan. A dose of 10 mg/kg (IP) decreased the bitings about 70% and a dose of 4 mg/kg (IP) about 50%.

Table 2 shows that the β-carbolines tested had different effects on phenylethylamine stereotypy. 6-Methoxyharmalan was the most effective, followed by β-carboline and 6-hydroxy-tetrahydro-β-carboline. Tetrahydro-β-carboline and its 6-methoxy- and 1-methyl derivatives were uneffective. 6-Methoxyharmalan was more potent than β-carboline, but their maximal effects were the same (Fig. 1).

DISCUSSION

None of the β-carbolines studied aggravated APO stereotypy but all inhibited it. It is not known if the major mechanism of β-carbolines on stereotyped behaviour caused by APO is partly mediated by direct action on dopaminergic system or if it is secondary to the increased 5HT action. Some other 5HT uptake inhibitors as well as the administration of precursor amino acids of 5HT and other procedures which increase brain 5HT concentration have decreased central dopaminergic action and increased the effects of neuroleptics in rats [10,34]. Dopaminergic and 5HT-ergic neurons have been shown to be in close connection at least in nucleus candatus [11, 22, 29].

The rank order of potency of different β-carbolines in the present study correlated well neither with the uptake inhibition of 5HT nor that of dopamine or noradrenaline [3,21]. The different effect of THBC and 6-OH-THBC may be partly due to differences in the ability to go through blood brain barrier. Some correlation seems to occur with the inhibition of MAO [9,14], so that the unsaturated 6-methoxyharmalan and β-carboline were more effective than the corresponding saturated tetrahydro-β-carbolines. More than one mechanism may occur. Only half of the substances tested inhibited also PEA stereotypy, but the mode of action on those cases may be similar. PEA is metabolized by MAO, but rather specifically by the type B. The fact that the effects of PEA never increased suggests that MAO-B is not significantly inhibited by the doses of β-carbolines used in vivo.

Dopamine receptors mediate APO stereotypy [4,12]. The major mechanism of amphetamine and PEA stereotypies have also been claimed to be dopaminergic. Although PEA releases dopamine from nerve terminals, the role of other effects of PEA in central nervous system is not excluded. Borison [7] has claimed that the actions of amphetamine and PEA are qualitatively identical, whereas some other studies suggest certain differences between them (for ref. see [7]). In light of our results, PEA and APO stereotypies are not identical, and β-carbolines did not inhibit them in the same way. Thus some direct effects of PEA or an effect on some other mediator than dopamine seem to be probable.

Although the concentrations of β-carbolines (THBC, 6-OH-THBC, 1-methyl-THBC) found in human body ([18, 20, 27], our unpublished results) are small in comparison with the doses of β-carbolines that show pharmacological activity in uptake processes or the doses used in animal experiments, nothing is known about their concentrations in the CNS synapses. This year Shoemaker *et al.* [31] reported a high concentration of harman (1-methyl-β-carboline) in rat arcuate nucleus. Thus the endogenic carbolines may have a role as regulators of 5HT and other transmitter substances. If the animal stereotypies used in the present study are valid models for human schizophrenic or paranoid psychoses, the β-carbolines formed in human body seem to be protecting against such psychoses rather than aggravating or inducing them.

ACKNOWLEDGEMENT

Supported in part by Sigrid Jusélius Foundation.

REFERENCES

1. Airaksinen, M. M., B. T. Ho, R. An and D. Taylor. Major pharmacological effects of 6-methoxytetrahydro-β-carboline, a drug elevating the tissue 5-hydroxytryptamine level. *Arzneimittel-forsch.* **28:** 42–46, 1978.
2. Airaksinen, M. M., J. T. Huang, B. T. Ho, D. Taylor and K. Walker. The uptake of 6-methoxy-1,2,3,4,-tetrahydro-β-carboline and its effect on 5-hydroxytryptamine uptake and release in blood platelets. *Acta pharmac. tox.* **48:** 375–380, 1978.
3. Airaksinen, M. M., H. Svensk, J. Tuomisto and H. Komulainen. Tetrahydro-β-carbolines: *in vitro* inhibition of serotonin and dopamine uptake by human blood platelets. *Acta pharmac. tox.* **46:** 299–304, 1980.
4. Andén, N. E., A. Rubenson, K. Fuxe and T. Hökfelt. Evidence for dopamine receptor stimulation by apomorphine. *J. Pharm. Pharmac.* **19:** 627–629, 1967.
5. Bidder, T. G., D. W. Shoemaker, H. G. Boettger, M. Evans and J. T. Cummins. Harman in human platelets. *Life Sci.* **25:** 157–164, 1979.
6. Borison, R. L., A. D. Mosnain and H. C. Sabelli. Biosynthesis of brain 2-phenylethylamine: influence of decarboxylase inhibitors and d-amphetamine. *Life Sci.* **15:** 1837–1848, 1974.
7. Borison, R. L., H. S. Havdala and B. I. Diamond. Chronic phenylethylamine stereotypy in rats: A new animal model for schizophrenia? *Life Sci.* **21:** 117–122, 1977 a.
8. Borison, R. L., F. Lemus, M. Reyes, H. S. Havdala and B. I. Diamond. Regional localization of 2-phenylethylamine in human brain. *Res Communs psychol. Behav.* **2:** 193–201, 1977 b.
9. Buckholtz, N. S. and W. O. Boggan. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: Structure-activity relationship and substrate specifity. *Biochem. Pharmac.* **26:** 1991–1996, 1977.
10. Carter, C. J. and C. J. Pycock. Possible importance of 5-hydroxytryptamine in neuroleptic-induced catalepsy in rats. *Br. J. Pharmac.* **60:** 267P, 1977.
11. Cools, A. R. An integrated theory of the aethiology of schizophrenia: Impairment the balance between certain, *in series* connected dopaminergic, serotonergic, and noradrenergic pathways within the brain. In: *On the Origin of Schizophrenic Psychoses*, edited by H. M. van Praag. Amsterdam: De Erven Bohn BV, 1975, pp. 58–80.
12. Costall, B. and R. J. Naylor. The role of telenchephalic dopaminergic systems in the mediation of apomorphine-stereotyped behavior. *Eur. J. Pharmac.* **24:** 8–24, 1973.
13. Fisher, E., B. Heller, H. Spatz and H. Reggiani. Phenylethylamine content of human urine and rat brain, its alterations in pathological conditions and after drug administration. *Experientia* **28:** 307–308, 1972.

14. Ho, B. T., W. M. McIsaac, K. E. Walker and V. Estevez. Inhibitors of monoamine oxidase: Influence of methyl substitution on the inhibitory activity of β-carbolines. *J. Pharm. Sci.* **57:** 269–274, 1968.
15. Ho, B. T. and K. E. Walker. 1,2,3,4-tetrahydro-β-carboline. *Org. Synth.* **51:** 136–138, 1971.
16. Ho, B. T., D. Taylor and K. E. Walker. Metabolism of 6-methoxytetrahydro-β-carboline in rats. *Xenobiotica* **2:** 349–362, 1972.
17. Ho, B. T., D. Taylor, K. W. Walker and W. M. McIsaac. The mode of action of 6-methoxy-1,2,3,4-tetrahydro-β-carboline on brain serotonin. *Can. J. Biochem.* **51:** 482–485, 1973.
18. Honecker, H. and H. Rommelspacher. Tetrahydronorharmane (tetrahydro-β-carboline) a physiologically occurring compound of indole metabolism. *Naunyn-Schmiedebergs Arch. Pharmac.* **305:** 135–141, 1978.
19. Inwang, E. E., A. D. Mosnaim and H. C. Sabelli. Isolation and characterization of phenylethylamine and phenylethanolamine from human brain. *J. Neurochem.* **20:** 1469–1473, 1973.
20. Kari, I., P. Peura and M. M. Airaksinen. Mass fragmentographic determination of tetrahydro-β-carboline in human blood platelets and plasma. *Med. Biol.* **57:** 412–414, 1979.
21. Komulainen, H., J. Tuomisto, M. M. Airaksinen, I. Kari, P. Peura and L. Pollari. Tetrahydro-β-carbolines and corresponding tryptamines: In vitro inhibition of serotonin, dopamine and noradrenaline uptake in rat brain synaptosomes. *Acta pharmac. tox.* **46:** 290–298, 1980.
22. Kostowski, W. Interactions between serotoninergic and catecholaminergic systems in the brain. *Pol. J. Pharmac. Pharm.* **27:** Suppl. 15, 1975.
23. Potkin, S. G., F. Karoum, L.-W. Chuang, H. E. Cannon-Spoor, I. Phillips and R. J. Wyatt. Phenylethylamine in paranoid chronic schizophrenia. *Science* **206:** 470–471, 1979.
24. Rommelspacher, H., P. Bade, H. Coper and G. Kossmehl. Inhibition of reuptake of serotonin by tryptoline. *Naunyn-Schmiedebergs Arch. Pharmac.* **292:** 93–95, 1976.
25. Rommelspacher, H., H. Kauffman, C. Heyck Conitz and H. Coper. Pharmacological properties of tetrahydronorharmane (tryptoline). *Naunyn-Schmiedebergs Arch. Pharmac.* **298:** 83–91, 1977.
26. Rommelspacher, H., S. Strauss and K. Rehse. β-Carbolines: A tool for investigating structure activity relationship of the high affinity uptake of serotonin, noradrenaline, dopamine, GABA and choline into a synaptosome-rich fraction of various regions from rat brain. *J. Neurochem.* **30:** 1573–1578, 1978.
27. Rommelspacher, H., H. Honecker, M. Barbey and B. Meinke. 6-Hydroxytetrahydronorharmane (6-hydroxy-tetrahydro-β-carboline) a new active metabolite of indole-alkylamines in man and rat. *Naunyn-Schmiedebergs Arch. Pharmac.* **310:** 35–41, 1979.
28. Sabelli, H. C. and W. J. Giardina. Amine modulation of affective behaviour. In: *Chemical Modulation of Brain Function*, edited by H. C. Sabelli. New York: Raven Press, 1973, pp. 225–229.
29. Samanin, R. and S. Garattini. The serotoninergic system in the brain and its possible functional connections with other aminergic systems. *Life Sci.* **17:** 1201–1209, 1975.
30. Sandler, M. and G. P. Reynolds. Does phenylethylamine cause schizophrenia? *Lancet* **1:** 70–71, 1976.
31. Shoemaker, D. W., J. T. Cummins, T. G. Bidder, H. G. Boettger and M. Evans. Identification of harman in the rat arcuate nucleus. *Naunyn-Schmiedebergs Arch. Pharmac.* **310:** 227–230, 1980.
32. Tuomisto, L. and J. Tuomisto. Inhibition of monoamine uptake in synaptosomes by tetrahydroharmane and tetrahydroisoquinoline compounds. *Naunyn-Schmiedebergs Arch. Pharmac.* **279:** 371–380, 1973.
33. Waldmeir, P. C. and A. Delini-Stula. Serotonin-dopamine interactions in the nigrostriatal system. *Eur. J. Pharmac.* **55:** 363–373, 1979.
34. Weiner, W. J., C. Goetz and H. L. Kiawans. Serotonergic and antiserotonergic influences of apomorphine induced stereotyped behavior. *Acta pharmac. tox.* **36:** 155–160, 1975.
35. Willner, J., H. F. Leferre and E. Costa. Assay by multiple ion detection of phenylethylamine and phenylethanolamine in rat brain. *J. Neurochem.* **23:** 857–858, 1974.
36. Wyatt, R. J., J. C. Gillin, D. M. Stoff, E. A. Moja and J. R. Tinklenberg. β-Phenylethylamine and the neuropsychiatric disturbances. In: *Neuroregulators and Psychiatric Disorders*. edited by E. Usdin, J. Barchas and D. Hamburg. New York: Oxford Univ. Press, 1977, pp. 31–45.