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Effects of 6-Methoxy-1,2,3,4-Tetrahydro-β-Carboline (6-MeO-THβC) on Audiogenic Seizures in DBA/2J Mice[1]

DAVID L. SPARKS* AND NEIL S. BUCKHOLTZ†

*†*Department of Biochemistry and* †*Department of Psychiatry and Behavioral Sciences*
Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29403 USA

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SPARKS, D. L. AND N. S. BUCKHOLTZ. *Effects of 6-methoxy-1,2,3,4,-tetrahydro-β*-carboline (6-MeO-THβC) on audiogenic seizures in DBA/2J mice. PHARMAC. BIOCHEM. BEHAV. **12**(1) 119–124, 1980.—It was found previously that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THβC) increased brain concentration of the neurotransmitter serotonin (5-HT) and decreased the concentration of its metabolite 5-hydroxyindole acetic acid (5-HIAA) at the same time the compound attenuated audiogenic seizures (AGS) in DBA/2J mice. In the present study we determined the time-course and dose-response effects of 6-MeO-THβC for blockade of AGS. Drugs sharing common effects with 6-MeO-THβC were also tested. At a dose of 100 mg/kg, 6-MeO-THβC blocked AGS between 10 min and 12 hr after injection, with maximal inhibition at 1 hr at which time a dose-related decrease in AGS was also demonstrated. All of the drugs tested which blocked AGS, including 6-MeO-THβC, THβC, 5-Hydroxytryptophan, chlorimipramine and pargyline, have biochemical similarities suggesting that facilitating serotonin function may be responsible for seizure-attenuating effects.

Audiogenic seizures Mice 6-methoxy-1,2,3,4-tetrahydro-β-carboline Serotonin

IT has previously been found that biogenic amine neurotransmitters have some role in the mediation of a variety of seizures [23]. In general, decreases in serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) concentration, whether naturally occurring or chemically induced [23], seem to increase seizure susceptibility (S-S) [23], whereas increases in 5-HT or NE concentration seem to protect against seizures [32,33]. These changes in concentrations of 5-HT and NE may be caused by changes in synthesis [21], degradative metabolism [21], presynaptic uptake [4], or vesicular uptake.

The DBA/2J mouse strain has been used as a model for determining the effects of various pharmacological compounds on seizures. The DBA/2J strain is genetically susceptible to sound-induced seizures (i.e. audiogenic seizures, AGS) at age 21 ± 5 days [31] and has a significantly lower concentration of 5-HT and NE only at ages of maximal susceptibility [31].

There is evidence which would indicate that 5-HT has a greater role in S-S than NE [23]. Buckholtz [3] found that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THβC) increased 5-HT concentration and decreased 5-hydroxyindoleacetic acid (5-HIAA, the deaminated metabolite of 5-HT) concentration at the same time blocking AGS in mice of the DBA/2J strain without affecting NE levels [24].

It was decided to extend the above findings on 6-MeO-THβC by determining a time and dose-effect relationship on inhibition of AGS and also to test the effects on AGS of other drugs sharing common neurochemical effects with 6-MeO-THβC. In this way we hoped to determine whether a correlation could be found between seizure blocking drugs and brain serotonergic activity in these mice.

METHOD

Subjects

DBA/2J mice 21 days old were used. They were bred in our facilities from parents obtained from Jackson Labs.

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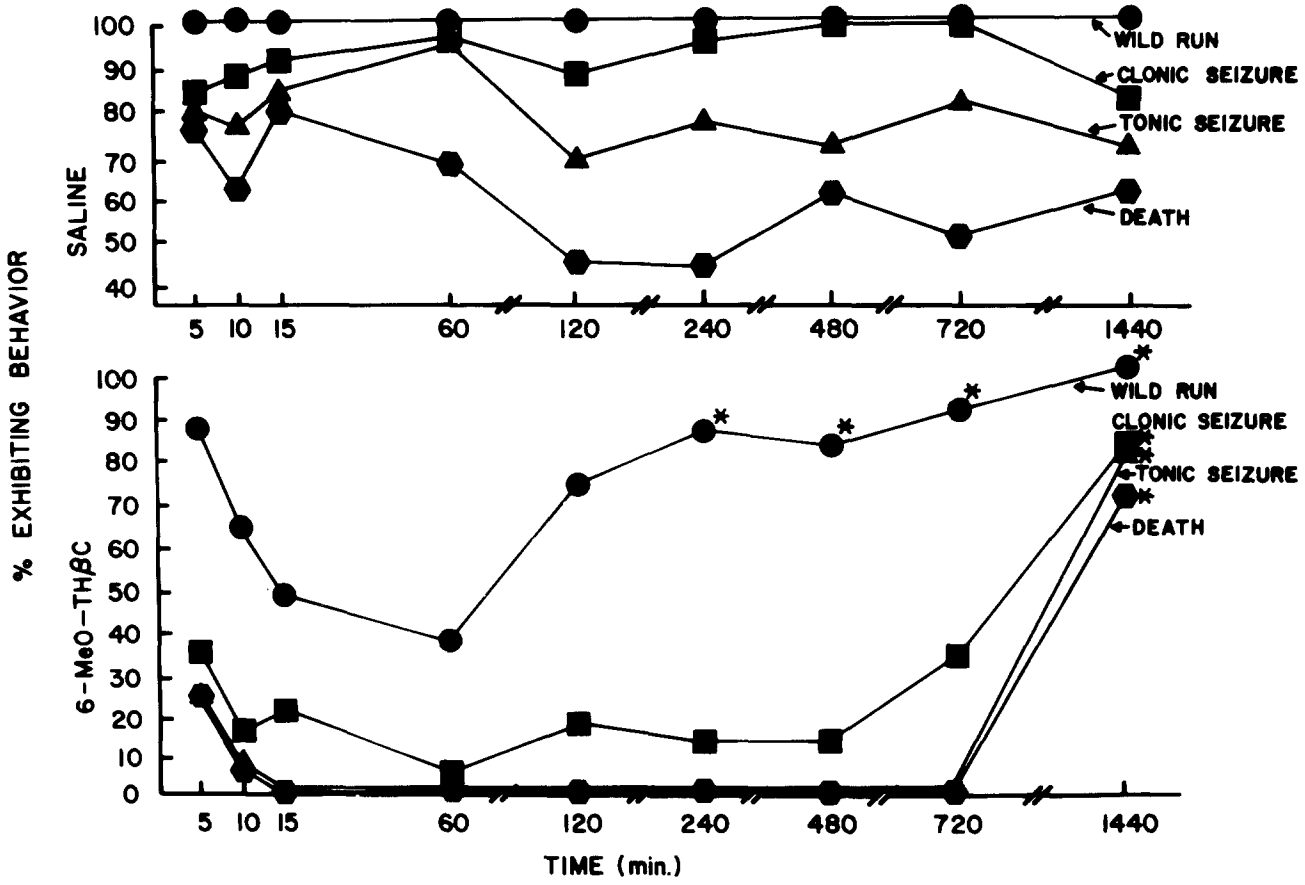

FIG. 1. Effects of saline (0.9%) (upper panel) or 6-MeO-THβC (100 mg/kg) (lower panel) on various components of AGS in 21 day old DBA/2J mice. Components of seizure are wild run, clonic seizure, tonic seizure and death. *Denotes non-significance, otherwise $p<0.0005$ for each component of seizure as compared to the same component of seizure in saline animals at the same time. Significance determined by Fisher exact probability.

Drugs and Reagents

Drugs which were purchased and their sources are as follows: 6-methoxy-tetrahydroharman, harman, 1,2,3,4-tetrahydro-β-carboline (THβC, as noreleagnine), norharman, 5-hydroxytryptophan (5-HTP), serotonin creatinine sulfate, 5-methoxy-N, N-dimethyltryptamine, from Sigma Chemical Company; and tetrahydroharman from ICN Pharmaceuticals. 6-Methoxy-tetrahydro-β-carboline HCl was synthesized according to the method of Ho *et al.* [15]. The following drugs were gifts from their respective sources: Fluoxetine (Lilly 110140, Eli Lilly), Quipazine (Miles Laboratories), Chlorimipramine (Ciba-Geigy Pharmaceutical Company), Lu 10-171 (H. Lundbeck and Company), pargyline (Abbott Labs), Clorgyline (May and Baker, Ltd., Dagenham, England) and Deprenyl (Dr. J. Knoll, Semmelweiss University, Budapest, Hungary). Doses of reference drugs were determined from the literature so that the maximal neurochemical effect was elicited.

Procedure

Injections of subjects. All injections were IP at a volume of 0.2 ml per 10 g body weight. All injections were given so that testing for AGS susceptibility occurred between 1030–1130 so as to control for circadian rhythms of 5-HT [34].

AGS testing. Subjects were removed from the colony and put six to a cage making sure to split up the litters so that each member of the litter received a different drug. After waiting the appropriate time after injection, subjects were placed, one at a time, into a bell jar 30.45 cm in dia. × 45.72 cm high. On top of the bell jar was a wooden cover with a bell (Edwards No. 13, generating 112 ± 2 db) which can be engaged from outside of the testing apparatus. The subject is allowed to become accustomed to the new environment for 15 sec. Then the bell is rung for 60 sec or until death is recorded. The scoring is as follows: wild run—where the subject runs wildly around the bottom of the bell jar, sometimes to the point of scaling the wall of the bell jar; clonic seizure—where the subject loses the righting reflex and flails all four limbs uncontrollably; tonic seizure—where the subject has all four limbs extended. Death is indicated by the ears standing up and no noticeable breathing. Latency of each of these behavioral events is recorded in seconds throughout the test period.

Statistical significance was determined by Fisher Exact Probability by comparing each component of seizure in drug

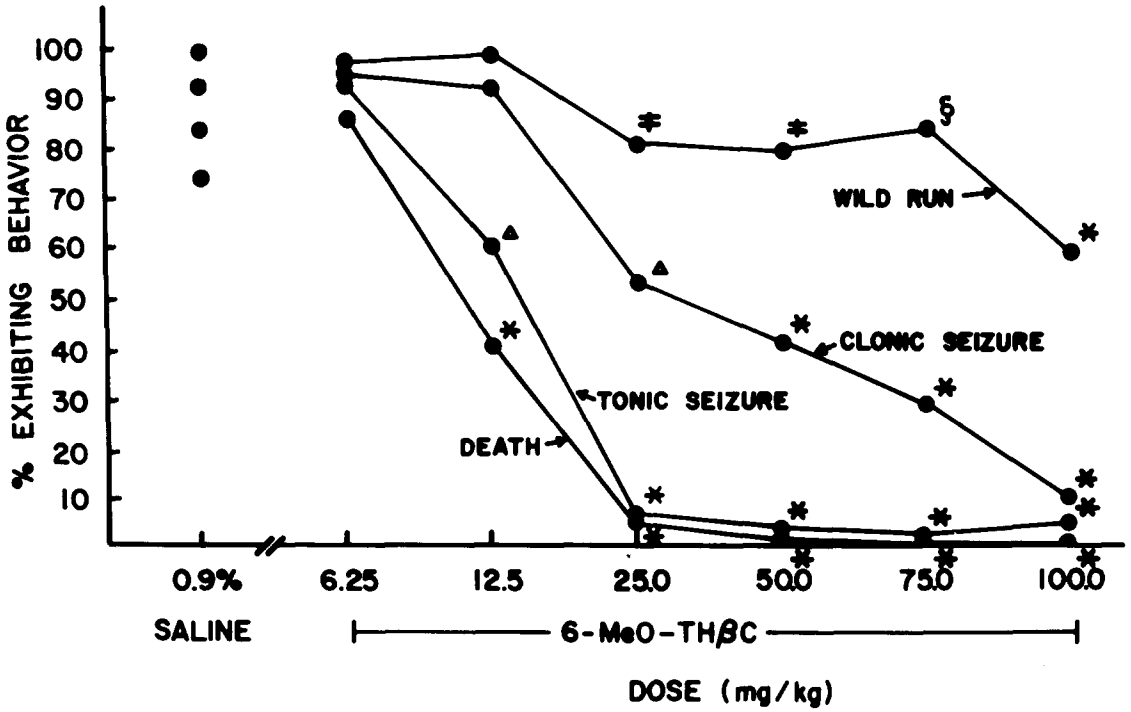

FIG. 2. Effect of saline or various doses of 6-MeO-THBC on components of AGS 1 hr post injection in 21 day old DBA/2J mice. Components of seizure are wild run, clonic seizure, tonic seizure and death. Significance, denoted as; §$p<0.05$, ‡$p<0.01$, △$p<0.001$ and *$p<0.0005$ for each component of seizure as compared to the same component of seizure in saline animals was determined by Fisher exact probability.

injected animals to the same component of seizure in saline animals [6].

RESULTS

At a 100 mg/kg dose, 6-MeO-THβC was effective at blocking AGS between 10 min and 12 hr after injection with maximal inhibition at 1 hr (Fig. 1). The dose-response effect at 1 hr showed that 6-MeO-THβC was effective at blocking AGS at doses between 25 and 100 mg/kg with a maximal effect of 100 mg/kg (Fig. 2). Other β-carbolines were also tested at one hour, at a dose of 50 mg/kg except THβC, for which a dose study was done. Their effectiveness at blocking AGS relative to 6-MeO-THβC (50 mg/kg) was as follows: THβC > 6-MeO-THβC > 6-MeO-tetrahydroharman > tetrahydroharman (Table 1).

Reference drugs with known actions were also tested and these results are shown in Table 1. The monoamine oxidase (MAO) inhibitor pargyline (inhibits both MAO A and B) effectively blocked AGS. Clorgyline (a proposed specific MAO-A inhibitor) [28], and Deprenyl (a proposed specific MAO-B inhibitor) [28], had little or no effect. The 5-HT uptake blocker chlorimipramine [38] blocked AGS but the uptake blockers fluoxetine (Lilly 110140) [20,38], and Lu 10-171 [17] had effects on only the tonic and death components of AGS. The serotonin receptor agonists were quipazine [22] which blocked only the death component, and 5-Methoxy-N-N-dimethyl-tryptamine [11] which had no effect on AGS. The serotonin precursor 5-HTP effectively blocked AGS.

DISCUSSION

The results show that 6-MeO-THβC and THβC block AGS in a time and dose related manner. The results also show that substitution at the C-1 position of the β-carboline nucleus decreases ability to block AGS (i.e. 6-MeO-tetrahydroharman vs. 6-MeO-THβC; tetrahydroharman vs. THβC; harman vs. norharman) and that C-6 substitution with a methoxy group can improve AGS blocking activity in the unsaturated β-carbolines (6-MeO-tetrahydroharman vs. tetrahydroharman).

Previous studies on 6-MeO-THβC have shown that neurochemically the drug is more potent in inhibiting 5-HT uptake than NE or DA uptake *in vitro* [30] and *in vivo* [4]. 6-MeO-THβC also inhibits the passive (4°C) uptake of 5-HT in a dose related manner (Sparks, unpublished observation) which may indicate that the drug is taken up into synaptosomes in place of 5-HT. 6-MeO-THβC increases brain 5-HT levels and decreases 5-HIAA levels [3] without affecting NE [24] or DA levels (Leroy Blank, unpublished observation). 6-MeO-THβC also blocks 5-HT oxidation by inhibiting MAO-A, which is specific for 5-HT and NE, with little effect on MAO-B ([25]; Sparks and Buckholtz, submitted). The fact that only 5-HT and 5-HIAA levels are affected would indicate only 5-HT specific MAO-A or MAO-A within 5-HT neurons is inhibited. Thus, neurobiochemically, 6-MeO-THβC seems to alter only 5-HT function, while blocking AGS in 21 day old DBA/2J mice. One could hypothesize that 6-MeO-THβC increases 5-HT at its post-synaptic recep-

TABLE 1

EFFECTS OF VARIOUS COMPOUNDS ON COMPONENTS OF AUDIOGENIC SEIZURES IN 21 DAY OLD DBA/2J MICE. ALL MICE WERE TESTED 60 MIN AFTER INJECTION

Drug	(N)	Dose (mg/kg)	% Exhibiting Behavior			
			Wild Run	Clonic	Tonic	Death
Saline	(125)	0.9%	100	98	96	80
β-Carbolines						
THβC	(25)	12.5	96	76†	56‡	40‡
THβC	(26)	25	92	61‡	12‡	11‡
THβC	(25)	50	88†	8‡	0‡	0‡
THβC	(26)	100	56‡	8‡	0‡	0‡
6-MeO-Tetrahydroharman	(38)	50	92	55‡	16‡	10‡
Tetrahydroharman	(10)	50	100	100	90	80
Harman	(7)	50	100	86	43‡	29‡
Norharman	(30)	50	80†	63‡	10‡	10‡
MAO Inhibitors						
Deprenyl	(30)	5	100	93	93	39‡
Deprenyl	(21)	10	100	100	100	24‡
Clorgyline	(30)	5	100	100	92	57*
Clorgyline	(22)	10	100	100	100	56*
Pargyline	(22)	50	77†	68‡	50‡	14‡
Pargyline	(20)	100	80†	65‡	30‡	15‡
Uptake blockers						
Chlorimipramine	(25)	25	88†	68‡	16‡	8‡
Fluoxetine (Lilly 110140)	(30)	10	100	93	64‡	33‡
LU 10- 171	(20)	10	100	100	65‡	55*
5-HT Receptor Agonists						
5-MeO-N,N-Dimethyltryptamine	(15)	10	100	100	98	78
Quipazine	(20)	10	100	100	100	16‡
Quipazine	(17)	20	100	100	76*	6‡
Other						
5-Hydroxy-Tryptophan	(20)	50	90	70‡	45‡	35‡
5-Hydroxy-Tryptophan	(20)	100	60‡	30‡	5‡	5‡

*$p<0.01$, †$p<0.005$, ‡$p<0.0005$ for each component of seizure as compared to the same component of seizure in saline animals.

tor and that this in turn is responsible for attenuation of AGS.

We have tried to determine if other drugs which alter 5-HT function in ways similar to 6-MeO-THβC also affect AGS in the same way. Drug studies reported here do not conclusively demonstrate that increasing 5-HT activity at its receptor blocks AGS but the data collected do seem to favor that hypothesis.

It is obvious that 6-MeO-THβC does not block AGS by acting as a 5-HT receptor agonist because the receptor agonists quipazine and 5-MeO-DMT do not block AGS. This does not exclude the possibility that only 5-HT acting at its receptor and not other putative agonists can block AGS. Although quipazine does increase 5-HT concentration slightly [9,19], both quipazine and 5-MeO-DMT could inhibit the binding of endogenous 5-HT by binding the receptor themselves [1, 36, 37].

The precursor to 5-HT, 5-HTP, when administered to 21 day old DBA/2J mice increases 5-HT levels [2], inhibits both MAO-A and 5-HT uptake (Sparks, unpublished observation) and blocks AGS. This would be evidence favoring the hypothesis but does not pinpoint any single mechanism leading to anti-AGS activity. It does possibly indicate that a combination of biochemical alterations may be necessary to attenuate AGS.

Chlorimipramine (CIMI), citalopram (LU10-171) and fluoxetine (Lilly 110140) are all competitive inhibitors of 5-HT uptake [16,39] and have little effect on the uptake of NE or DA [4, 18, 38]. Their order of potency is not consistent in all published studies, but most recent data indicate CIMI > fluoxetine ≥ citalopram [4, 5, 16]. To fit the hypothesis all of these drugs should block AGS, but only CIMI does effectively. CIMI is the only one of these uptake blockers shown to increase 5-HT levels [8, 14, 18] and to inhibit MAO-A measured *in vivo* in 21 day old DBA/2J mice, with none of them affecting MAO-B (Sparks, unpublished observation). CIMI may also increase release of 5-HT [12]. Thus it seems possible that CIMI attenuates AGS via an increase of

5-HT at its receptor which occurs by MAO-A inhibition, 5-HT release and 5-HT uptake blockade. Once again, it seems that a combination of 5-HT biochemical alterations may be necessary for total seizure blocking activity, in that citalopram and fluoxetine only partially block AGS.

The "specific" MAO inhibitors clorgyline for MAO-A and deprenyl for MAO-B [28] did not block AGS, but pargyline, which inhibits both MAO-A and B at the dose used [7,10] does block AGS. Keeping in mind that MAO-B has been postulated to function as a metabolizing enzyme for extraneural neurotransmitters [27], pargyline may block AGS by increasing 5-HT via inhibition of MAO-A with subsequent 5-HT "spill over" [13], and decreased metabolism of extraneural 5-HT by inhibiting MAO-B [29].

All of these data on pharmacological manipulation of AGS in DBA/2J mice are analogous to data gathered on the proposed specific 5-HT hyperactivity model in rats and mice [13, 26, 35] which indicates that the same type of drug manipulations which produce hyperactivity by increasing 5-HT at its receptor may also block AGS in 21 day old DBA/2J mice by a similar mechanism. Thus, by correlation, AGS in DBA/2J mice may also be a useful model for assessing the functional consequences of 5-HT manipulations.

REFERENCES

1. Bennett, J. P. and S. H. Snyder. Sterospecific binding of D-Lysergic acid Diethylamide (LSD) to brain membranes: relationship to serotonin receptors. *Brain Res.* **94:** 523–544, 1975.
2. Boggan, W. O. and L. S. Seiden. 5-Hydroxytryptophan reversal of reserpine enhancement of audiogenic seizure susceptibility in mice. *Physiol. Behav.* **10:** 9–12, 1973.
3. Buckholtz, N. S. Anti-convulsant effects of 6-methoxy-1,2,3,4-tetrahydro-β-carboline on audiogenic and electroconvulsive seizures in mice. *Pharmac. Biochem. Behav.* **8:** 54–68, 1975.
4. Buckholtz, N. S. and W. O. Boggan. Inhibition by β-carbolines of monoamine uptake into a synaptosomal preparation: Structure-activity relationships. *Life Sci.* **20:** 2093–2100, 1977.
5. Davis, P. A., J. Baird-Lambert, K. M. Taylor and J. A. MacLaren. Serotonergic activity of a novel tetrahydro-β-carboline. *Biochem. Pharmac.* **28:** 1803–1806, 1978.
6. Fisher, R. A. *Statistical Methods for the Research Worker*, 5th Ed. Edinburgh: Oliver and Boyd, 1934.
7. Fuentes, J. A., J. Garzon and J. DelRio. Potentiation of morphine analgesia in mice after inhibition of brain type B monoamine oxidase. *Neuropharmacology* **16:** 857–862, 1977.
8. Fuller, R. W., K. W. Perry and B. B. Molloy. Effect of an uptake inhibitor on serotonin metabolism in rat brain: Studies with 3-(p-trifluoromethyl phenoxy)-N-Methyl-3-phenylpropylamine (Lilly 110140). *Life Sci.* **15:** 1161–1171, 1974.
9. Fuller, R. W., H. D. Snoddy, K. W. Perry, B. W. Roush, B. B. Molloy, F. P. Bymaster and D. T. Wong. The effects of Quipazine on 5-HT metabolism in rat brain. *Life Sci.* **18:** 925–934, 1976.
10. Fuller, R. W. and S. K. Hemrick. Inability of imipramine to protect against type A or type B monoamine oxidase inhibition by pargyline. *Res. Communs chem. pathol. Pharmac.* **23:** 411–414, 1979.
11. Fuxe, K., B. Holmstedt and G. Jonsson. Effects of 5-methoxy-N-N-dimethyl tryptamine on central monoamine neurons. *Eur. J. Pharmac.* **19:** 25–34.
12. Gershon, M. D. and G. M. Jonakait. Uptake and release of 5-HT by enteric 5-Hydroxytryptaminergic neurons: Effects of Fluoxetine (Lilly 110140) and chlorimipramine. *Br. J. Pharmac.* **66:** 7–9, 1979.
13. Grahame-Smith, D. G. Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.* **18:** 1053–1066, 1971.
14. Halaris, A. E., R. A. Lovell and D. X. Freedman. Effect of chlorimipramine on the metabolism of 5-HT in the rat brain. *Biochem. Pharmac.* **22:** 2200–2202, 1973.
15. Ho, B. T., W. M. McIsaac, K. E. Walker and V. Estevez. Inhibitors of monoamine oxidase. *J. Pharm. Sci.* **57:** 269–278, 1968.
16. Hyttel, J. Effect of a specific 5-HT uptake inhibitor, Citalopram (Lu10-171), on ^{3}H-5-HT uptake in rat brain synaptosomes in vitro. *Psychopharmacology* **60:** 13–18, 1978.
17. Hyttel, J. Neurochemical characterization of a new potent and selective serotonin uptake inhibitor: Lu10-171. *Psychopharmacology* **51:** 225–233, 1977.
18. Hyttel, J. Effect of a selective 5-HT uptake inhibitor-Lu10-171 on rat brain 5-HT turnover. *Acta. pharmac. tox.* **40:** 439–446, 1977.
19. Jacoby, J. H., R. A. Howd, M. S. Levin and R. J. Wurtman. Mechanisms by which Quipazine, a putative serotonin receptor agonist, alters brain 5-Hydroxyindole metabolism. *Neuropharmacology* **15:** 529–534, 1976.
20. Kellar, K. J., G. R. Elliott, R. B. Holman, J. Vernikos-Danellis and J. D. Barchas. Tryptoline inhibition of serotonin uptake in rat forebrain homogenates. *J. Pharmac. exp. Ther.* **198:** 619–625, 1976.
21. Kellogg, C. Serotonin metabolism in the brains of mice sensitive or resistant to audiogenic seizures. *J. Neurobiol.* **2:** 209–214, 1971.
22. Malick, J. B., E. Foren and A. Barnett. Quipazine-induced head twitch produced by serotonin receptor activation in mice. *Pharmac. Biochem. Behav.* **6:** 325–329, 1977.
23. Maynert, E. W., T. S. Marczynski and R. A. Browning. The role of the neurotransmitters in the epilepsies. *Advances in Neurology*, Vol. 13. New York: Raven Press, 1975, pp. 79–148.
24. McIsaac, W. M., D. Taylor, E. E. Walker and B. T. Ho. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline—a serotonin elevator. *J. Neurochem.* **19:** 1203–1206, 1972.
25. Meller, E., E. Friedman, J. W. Schweitzer and A. S. Friedhoff. Tetrahydro-β-carbolines: Specific inhibitors of Type A monoamine oxidase in rat brain. *J. Neurochem.* **28:** 995–1000, 1977.
26. Modigh, K. Effects of chlorimipramine and protriptyline on the hyperactivity induced by 5-hydroxytryptophan after peripheral decarboxylase inhibition in mice. *J. Neural Trans.* **34:** 101–109, 1973.
27. Murphy, D. L. Substrate-selective monoamine oxidases—inhibitor, tissue, species and functional differences. *Biochem. Pharmac.* **27:** 1889–1893, 1978.
28. Neff, N. H. and H.-Y. Yang. Another look at the monoamine oxidases and the MAO inhibitor drugs. *Life Sci.* **14:** 2051–2074, 1974.
29. Prozialeck, W. C. and W. H. Vogel. Deamination of 5-methoxytryptamine, serotonin and phenylethylamine by rat MAO *in vitro* and *in vivo*. *Life Sci.* **22:** 561–570, 1977.
30. Rommelspacher, H., S. M. Strauss and K. Rehse. β-Carbolines: A tool for investigating structure-activity relationships 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, 1977.
31. Schlesinger, K., W. O. Boggan and D. X. Freedman. Genetics of audiogenic seizures: I. Relation to brain serotonin and norepinephrine in mice. *Life Sci.* **4:** 2345–2351, 1965.

32. Schlesinger, K., W. O. Boggan and D. X. Freedman. Genetics of audiogenic seizures: II. Effects of pharmacological manipulation of brain serotonin, norepinephrine and gamma-aminobutyric acid. *Life Sci.* **7:** 437–447, 1968.
33. Schlesinger, K., W. O. Boggan and D. X. Freedman. Genetics of audiogenic seizures: III. Time response relationships between drug administration and seizure susceptibility. *Life Sci.* **9:** 721–729, 1970.
34. Schreiber, R. A. and K. Schlesinger. Circadian rhythms and seizure susceptibility: Effects of manipulation of light cycles on susceptibility to audiogenic seizures and on levels of 5-HT and NE in brain. *Physiol. Behav.* **8:** 699–703, 1972.
35. Squires, R. F. and J. Buus Lassen. The inhibition of A and B forms of MAO in the production of a characteristic behavioral syndrome in rats after L-tryptophan loading. *Psychopharmacology* **41:** 145–151, 1975.
36. Whitaker, P. M. and P. Seeman. High-affinity ^{3}H-serotonin binding to caudate: Inhibition by hallucinogens and serotoninergic drugs. *Psychopharmacology* **59:** 1–5, 1978.
37. Whitaker, P. M. and P. Seeman. Selective labeling of serotonin receptors by d- [^{3}H] lysergic acid diethylamide in calf caudate. *Proc. Natn Acad. Sci. USA* **75:** 5783–5787, 1978.
38. Wong, D. T., J. S. Horng, F. D. Bymaster, K. L. Hauser and B. B. Molloy. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluromethylphenoxy)-n-methyl-3-phenyl-propylamine. *Life Sci.* **15:** 471–479, 1974.
39. Wong, D. T., F. P. Bymaster, J. S. Horng and B. B. Molloy. A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(p-trifluoro-methylphenoxy)-N-methyl-3-phenyl propylamine. *J. Pharmac. exp. Ther.* **193:** 804–811, 1975.