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Anticonvulsant Effects of 6-Methoxy-1,2,3,4-tetrahydro-β-carboline on Audiogenic and Electroconvulsive Seizures in Mice[1]

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BUCKHOLTZ, N. S. *Anticonvulsant effects of 6-methoxy-1,2,3,4-tetrahydro-β-carboline on audiogenic and electroconvulsive seizures in mice.* PHARMAC. BIOCHEM. BEHAV. 3(1) 65–68, 1975. – 6-Methoxy-1,2,3,4,-tetrahydro-β-carboline (6-MeO-THBC) was tested for anticonvulsant properties against audiogenic seizures in DBA/2J and primed C57BL/6J mice (i.e., mice given a prior auditory exposure) and against electroconvulsive seizures in DBA/2J mice. 6-MeO-THBC (100 mg/kg) was found to attenuate both types of behavioral seizures 2 hr after injection as compared to saline controls. In addition, 6-MeO-THBC increased whole brain serotonin and decreased whole brain 5-hydroxyindoleacetic acid 2 hr after injection. These results support previous reports which suggest a serotonergic involvement in behavioral seizures.

Audiogenic seizures Electroconvulsive seizures 6-Methoxy-1, 2, 3, 4-tetrahydro-β-carboline Serotonin 5-Hydroxyindoleacetic acid

THE pharmacological modification of neurotransmitter systems has been a useful method for studying the role of neurotransmitters in behavior. A behavior which has been studied extensively in this way is seizures, and the pharmacological manipulation of seizures has shown that there is an involvement of the biogenic amines in this behavior [2, 11, 12]. In general, decreases in the biogenic amines increase behavioral seizure severity and increases in biogenic amines decrease seizure severity. Although this involvement has been shown for both the catecholamines norepinephrine and dopamine and the indoleamine serotonin or 5-hydroxytryptamine (5HT), the present paper deals specifically with 5HT.

In this regard, depletion of brain 5HT by parachlorophenylalanine [1, 18, 19] or tetrabenazine [18], and elevation of 5HT by 5-hydroxytryptophan [4, 16, 18, 19] or monoamine oxidase inhibitors [10, 18, 19] will, respectively, increase and decrease susceptibility to a variety of seizure inducing stimuli.

Recently, McIsaac *et al.* [13] reported that the drug 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) specifically increases brain 5HT without affecting norepinephrine. In order to assess the behavioral effects of this new drug, we chose to examine seizures since this behavior has been shown to be affected by manipulation of serotonin as discussed above.

METHOD

Animals

Inbred mice of the DBA/2J and C57BL/6J strains were bred in our laboratory from stocks obtained from the Jackson Laboratory, Bar Harbor, ME. Mice of both sexes in approximately equal number were tested at either 21 or 26 days of age. Mice were maintained under standard laboratory conditions (light on 0700, light off 1900) and remained in the breeding cages until tested. They were allowed ad lib access to Wayne Mouse Breeder Blox and tap water. All testing was done between 1100 and 1300 to control for circadian rhythms known to exist for both 5HT and seizures [21] and no mouse was used more than once.

Procedure

Design. Mice in each experiment were assigned either to the control or experimental groups. 6-MeO-THBC was dissolved in 0.9% saline (5 mg/ml; 0.02 ml/g), and the experimental group received 100 mg/kg 6-MeO-THBC i.p.

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The control group received the 0.9% saline vehicle (0.02 ml/g). The injection was given 2 hr before testing or sacrifice. The dose and time were chosen from prior data [13] as being maximally effective in elevating brain 5HT. 6-MeO-THBC was studied for its effects on the following: (1) Audiogenic seizures (AGS) in DBA/2J mice at 21 days of age, which is the age of maximum susceptibility [17]; (2) Electroconvulsive seizures in DBA/2J mice at 21 days of age; (3) AGS in C57BL/6J mice which were primed (see below) at 19 days and tested for AGS at 26 days; (4) AGS in C57BL/6J mice which were primed at 16 days and tested for AGS at 21 days; (5) Brain levels of 5HT and 5-hydroxyindole acetic acid (5HIAA) in DBA/2J and C57BL/6J (primed 16–21) mice 21 days old.

Testing procedure. For the AGS test, each mouse was removed from its cage, placed individually into a 45.72 cm high by 30.45 cm wide chromatography jar enclosed in a sound attenuation chamber, and allowed to adapt for 15 sec. A 7.62 cm electric bell (Edwards #13) generating 112 ± 2 db at the level of the mouse was then sounded for 60 sec, during which the incidence of wild running, clonic, tonic, and lethal seizures was recorded. An animal must pass through a wild run stage prior to a clonic seizure, and a clonic seizure prior to a tonic, and a tonic seizure prior to death. The data are presented to reflect this; i.e. if an animal manifested a tonic seizure it is also represented in the percent of animals in the group that wild ran and in the group that had clonic seizures.

Audiogenic priming refers to the fact that a C57BL/6J mouse not normally susceptible to AGS can be made susceptible by a prior exposure to the bell which, in itself, does not produce a seizure [6]. Priming was accomplished by placing each mouse in the jar and sounding the bell for 60 sec. No mouse that evidenced any components of an AGS during priming was used for subsequent testing. C57BL/6J mice were either primed at 19 days and tested at 26 days (19–26) or primed at 16 days and tested at 21 days (16–21). The 16–21 schedule produces a higher percentage of tonic seizures than does the 19–26 schedule [20]. No DBA/2J mouse in any of these experiments was ever primed.

Electroconvulsive shock (ECS) was given transcorneally by an apparatus [5] which consisted of an 800 V transformer with a fixed resistance of 160,000 ohms in series with the mouse to produce a current of 5 mA r.m.s. An electromechanical timer was used to obtain an ECS duration of 300 msec. The seizure was scored as either clonus, tonus, or death.

5HT and 5HIAA assays. Fluorimetric assays of whole brain 5HT and 5HIAA were done according to the method of Miller *et al.* [14]. Standards and reagents were supplied from a kit (No. 190020) commercially available from Regis Chemical Co., Morton Grove, IL. Data were corrected for recoveries of 80% and 85% for 5HT and 5HIAA respectively. Separate groups of mice were used for the behavioral and biochemical studies to avoid confounding the biochemical effects of the drug with any biochemical changes related to the seizure itself.

RESULTS

In each seizure experiment, each component of the behavioral seizure in the experimental group was compared with the same component in the control group. These comparisons were made by a 2×2 χ^2 except when the expected cell frequencies were less than 5 in which case the Fisher Exact Probability Test was used [22].

The major protective effect afforded by 6-MeO-THBC was on tonic seizures and death (Tables 1 and 2). In the DBA/2J mice tested for AGS, 6-MeO-THBC afforded protection of all components except wild running, and in the DBA/2J mice given ECS, protection was afforded against tonic seizures and death. The high incidence of death in 21 day old DBA/2J mice given ECS (5 mA, 300 msec) is not unreasonable since Schlesinger *et al.* [16], using the same mouse strain and age, found an ED50 for clonic-tonic seizures of 4.6 mA with a lower ECS duration (200 msec). The primed C57BL/6J mice showed a very low incidence of death so the major effect of 6-MeO-THBC in these mice was on tonic seizures, although in the mice primed on the 19–26 schedule there was protection of wild running.

6-MeO-THBC significantly increased brain 5HT and decreased brain 5HIAA in primed C57BL/6J (16–21) and in DBA/2J mice (Table 3).

DISCUSSION

The fact that 6-MeO-THBC increased brain 5HT and also effectively attenuated behavioral seizures is in agreement with previous data showing a serotonergic involvement in seizures. Depletion of brain serotonin in mice by reserpine increases audiogenic [18] electroconvulsive and metrazol-induced seizures [16]. Administration of 5HTP, which increases brain 5HT, decreases audiogenic [18,19], electroconvulsive [16] and metrazol-induced seizures [4,16]. Administration of 5HTP also reverses the reserpine-induced enhancement of AGS [3]. Thus, the behavioral effect of 6-MeO-THBC in decreasing seizure severity is consonant with its neurochemical effect of increasing brain 5HT. Similar behavioral and biochemical actions of 5HTP and 6-MeO-THBC have also been reported by Nance and Kilbey [15] who found that both compounds reversed the effects of parachlorophenylalanine on brain 5HT and on sucrose preference.

6-MeO-THBC produced a somewhat greater increase in brain 5HT in DBA/2J mice (233% of the control value) than in C57BL/6J mice (182% of the control value). This may indicate a more labile 5HT pool in DBA/2J mice. Additional pharmacological evidence suggesting a difference in 5HT metabolism between the two strains has been provided by Schlesinger *et al.* [18]. They reported that reserpine and tetrabenazine produced a greater depletion of 5HT in DBA/2J than in C57BL/6J mice, and the rate of repletion of 5HT after tetrabenazine was slower in DBA/2J than in C57BL/6J mice.

Non-pharmacological evidence for a serotonergic involvement in seizures was given by Schlesinger *et al.* [17] who reported that at 21 days of age, DBA/2J mice, which are naturally audiogenic seizure susceptible, had a lower level of brain 5HT than C57BL/6J mice, which are not normally susceptible at that age. Kellogg [9] also found a lower level of 5HT in brains of DBA/2J mice compared to C57BL/6J mice and in addition reported a faster rate of production of 5HT and a faster rate constant of disappearance of 5HT and 5HIAA in DBA/2J mice as compared to C57BL/6J mice. In the present experiment there was no difference in brain 5HT between the C57BL/6J and DBA/2J mice. The levels of 5HT in the C57BL/6J mice were comparable to those reported previously [9,17] but the levels in the DBA/2J

TABLE 1

THE EFFECT OF 6-MeO-THBC (100 mg/kg) OR SALINE INJECTIONS ON MICE TESTED 2 HR LATER FOR EITHER AUDIOGENIC OR ELECTROCONVULSIVE SEIZURES*

	6-MeO-THBC					Saline				
	N	%WR	%C	%T	%D	N	%WR	%C	%T	%D
DBA/2J – AGS	15	93	47	0	0	15	100	87	87	87
DBA/2J – ECS	12	–	100	0	0	12	–	100	100	83
C57BL/6J (19–26) – AGS	16	69	44	0	0	17	100	47	29	24
C57BL/6J (16–21) – AGS	15	73	47	0	0	16	88	69	69	12

*WR, wild run or more severe seizure; C, clonic seizure or more severe; T, tonic seizure or more severe; D, death.

TABLE 2

PROBABILITY VALUES FOR A TEST OF THE DIFFERENCE BETWEEN 6-MeO-THBC VS SALINE GROUPS FOR EACH COMPONENT OF SEIZURES

	WR	C	T	D
DBA/2J – AGS	NS	<0.05†	<0.0005†	<0.0005†
DBA/2J – ECS	–	NS	<0.0005†	<0.0005†
C57BL/6J (19–26) – AGS	<0.02*	NS	<0.03*	<0.06*
C57BL/6J (16–21) – AGS	NS	NS	<0.0005†	NS

*Fisher exact probability test
†χ^2, $df = 1$

TABLE 3

MEAN WHOLE BRAIN 5HT AND 5HIAA LEVELS (μg/g) ±S.D. IN 21 DAY OLD MICE KILLED 2 HR AFTER EITHER AN INJECTION OF 6-MeO-THBC (100 mg/kg) OR SALINE. SYMBOLS REFER TO PROBABILITY VALUES FROM A *t*-TEST BETWEEN 6-MeO-THBC AND SALINE GROUPS. (NUMBERS IN PARENTHESES DENOTE SAMPLE SIZE)

	6-MeO-THBC		Saline	
	5HT	5HIAA	5HT	5HIAA
C57BL/6J (16–21)	1.224 ± 0.124 (7)‡	0.155 ± 0.066 (7)†	0.674 ± 0.049 (6)	0.298 ± 0.086 (6)
DBA/2J	1.610 ± 0.239 (9)‡	0.238 ± 0.116 (9)*	0.691 ± 0.248 (8)	0.401 ± 0.103 (8)

*$p<0.02$; †$p<0.01$; ‡$p<0.001$

mice were higher. The present experiment, however, was not specifically designed to compare C57BL/6J and DBA/2J mice directly, so the biochemical analyses on the two strains were not done concurrently. Since the assays were performed several weeks apart, direct comparisons between the two strains may not be valid because of changes in such variables as circadian rhythm. This would not have affected the results within a strain, however, since the drug and control groups were assayed concurrently. The C57BL/6J mice in the present experiment had also been primed whereas the mice in previous studies [9,17] were not. However, Boggan (personal communication) found no difference in brain 5HT between primed and non-primed C57BL/6J mice. Kellogg [9] reported a higher level of 5HIAA in 21 day old DBA/2J mice as compared to C57BL/6J mice which approached statistical significance. In the present experiment, the difference in 5HIAA was in the same direction (Table 3) as that shown by Kellogg [9] and also approached statistical significance. ($t = 1.84$, $df = 12$, $p<0.10$).

The mechanism of action of 6-MeO-THBC in increasing brain 5HT has been investigated by Ho and colleagues. Ho *et al.* [8] found that 6-MeO-THBC increased peripheral 5-HTP decarboxylase. They suggested that this could increase blood 5HT which, if sufficiently elevated, would produce a concentration gradient from the blood to the brain. The present finding of a decrease in 5HIAA is in contrast to the results of McIsaac, *et al.* [13] who found no effect of 6-MeO-THBC on 5HIAA. This led them to conclude that the elevation of 5HT was not the result of monoamine oxidase (MAO) inhibition especially since a previous report [7] showed that 6-MeO-THBC was only a very weak inhibitor of MAO and since there was no increase in norepinephrine which might be expected if 6-MeO-THBC were acting via MAO inhibition. The explanation for the depletion of 5HIAA seen in our lab and not in that of Ho *et al.* [8] is presently unknown although the ages and strains of mice used were different. Further investigation with the compound will hopefully clarify the possible mechanisms among which are MAO inhibition, increased particulate binding of 5HT, and decreased uptake of 5HT into nerve endings thus making 5HT unavailable to MAO.

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