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# Effect of Cycloheximide and *d*-Amphetamine on Brain Catecholamines in Two Mouse Strains[1]

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CARR, L. A. AND S. M. WEHRY. *Effect of cycloheximide and* d-*amphetamine on brain catecholamines in two mouse strains*. PHARMAC. BIOCHEM. BEHAV. 13(2) 193–197, 1980.—The ability of cycloheximide to inhibit brain catecholamine synthesis in C57BL/6J and DBA/2J mice was studied to determine whether differences exist in these two strains with regard to this action and whether such effects correlate with previously reported differences in sensitivity to the amnesic effects. Administration of cycloheximide caused a dose-dependent inhibition of norepinephrine and dopamine synthesis in both strains. There was a significant effect due to strain on dopamine synthesis in drug-treated animals. *d*-Amphetamine partially prevented the decrease in the rate of synthesis of norepinephrine, dopamine and normetanephrine caused by cycloheximide in the C57 strain but enhanced the inhibition of synthesis of these compounds in the DBA strain. The results suggest that the reported differences in sensitivity to the behavioral effects of cycloheximide may be associated with the degree of inhibition of catecholamine synthesis in these two mouse strains.

Cycloheximide Norepinephrine Dopamine *d*-Amphetamine Mouse strains Memory

SEVERAL antiobiotics, including cycloheximide and acetoxycycloheximide, have been used extensively in the study of mechanisms involved in the formation of long-term memory [6]. Since these agents are potent inhibitors of cerebral protein synthesis, it has been proposed that the disruptive effects of these drugs on long-term memory formation result from a deficiency of specific proteins which are involved in the formation of memory traces [26]. However, evidence from several recent studies suggest that additional mechanisms, such as disruption of neurotransmitter function, may underlie the impairment of memory consolidation or retention. For example, cycloheximide has been shown to inhibit the accumulation of newly synthesized catecholamines [3,10]. Furthermore, noradrenergic agonists, such as norepinephrine, clonidine and isoproterenol [13,19], and drugs which are believed to enhance central catecholamine neuron activity, such as *d*-amphetamine [18,20], prevent or reverse the amnesic effects of cycloheximide. This latter action appeared to correlate with an attenuation of the cycloheximide-induced decrease in catecholamine synthesis rather than alteration of protein synthesis inhibition [3].

Various mouse strains exhibit differences in behavior, in their ability to learn specific tasks and in their sensitivity to the amnesic effects of cycloheximide. The C57BL/6J strain, which is characterized by high levels of spontaneous motor activity [15] and low levels of avoidance learning [4], is much less resistant to the amnesic effects of cycloheximide [22] when compared with the DBA/2J strain. This difference in strain sensitivity has been attributed to possible differences in the duration or degree of inhibition of protein synthesis [11,22] or in the pharmacokinetics of the drug [28].

The aim of the present study was to examine the effects of cycloheximide on the synthesis of brain norepinephrine and dopamine in these two strains and to determine whether these effects correlate with the reported differences in behavioral sensitivity to this drug. It was also of interest to determine whether *d*-amphetamine interacted with cycloheximide in a different fashion in these two strains with respect to catecholamine synthesis and metabolism.

## METHOD

Male mice of two strains, C57BL/6J and DBA/2J (Jackson Labs), between 10 and 14 weeks old and weighing 15–25 g were used in this study. They were housed in small groups of 5–6 animals and provided with water and Purina Rat Chow ad lib. One hour prior to treatment, between 10 a.m. and 12 p.m., the mice were moved to the laboratory and placed in individual cages.

In Experiment 1, various doses of cycloheximide (Sigma) were administered subcutaneously on the back of the neck to mice of both strains one hour before sacrifice. Fifteen minutes prior to sacrifice by cervical dislocation, 100 $\mu$Ci of 3,5 $^3$H-tyrosine (40–60 mCi/mmole, New England Nuclear) in

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0.2 ml phosphate buffered saline (0.14 M NaCl, 0.01 M $NaPO_4$, pH 7.0) were injected intravenously via the dorsal tail vein. Following decapitation, the brain was removed and after dissecting away the cerebellum, olfactory bulbs and lower brain stem, the remaining tissue was weighed and homogenized in 3 ml ice cold 0.4 N $HClO_4$. After centrifugation and rehomogenization [17] the tissue supernate was frozen until assayed. Endogenous tyrosine was measured fluorometrically by the method of Waalkes and Udenfriend [29]. The concentrations of $^3$H-tyrosine, $^3$H-norepinephrine, and $^3$H-dopamine were measured in the brain homogenates by methods previously reported [3]. The approximate recoveries for these compounds were 90, 40 and 30 percent, respectively. The concentration of all labeled compounds was corrected for recovery. The relative rates of catecholamine synthesis were calculated by dividing the brain concentration of $^3$H-catecholamine by the specific activity of tyrosine [25]. The incorporation of $^3$H-tyrosine into brain protein was determined from the radioactivity in the tissue pellet and supernate derived from the centrifugations above. After washing the pellet and digesting in 0.2 NaOH for 2 days [3] the tyrosine incorporation ratio was calculated by dividing the radioactivity in the pellet by the radioactivity in the supernate.

In Experiment 2, mice of both strains received one of four drug treatments: (1) saline+saline, (2) saline+*d*-amphetamine sulfate (Smith, Kline, and French), (3) cycloheximide+saline, (4) cycloheximide+*d*-amphetamine sulfate according to the schedule given in Table 1. 100 $\mu$Ci of $^3$H-tyrosine were administered intravenously 15 min before sacrifice. The brains from these animals were assayed for $^3$H-catecholamines and their rates of accumulation were determined as described above. In addition, the rates of formation of their 0-methylated metabolites, normetanephrine (NM) and 3-methoxytyramine (3-MT) were also determined according to the following procedure. After shaking the tissue supernate with alumina [3], the supernate was removed and adjusted to pH 6.5 with 3 M Tris buffer and added to an Amberlite CG-50 column (200–400 mesh, 4 cm) which had been washed previously with 15 ml sodium phosphate buffer, pH 6.5, containing 1% EDTA. After washing the column with 20 ml $H_2O$, the 0-methylated metabolites were eluted with 5 ml of 1 N HCl. The eluate was adjusted to pH 6.5 with 2 N NaOH and added to a Dowex 50W× 4 column (200–400 mesh, $Na^+$ form, 4 cm). After washing the column with 10 ml $H_2O$ and 6 ml of 2 N HCl, $^3$H-NM was eluted with 10 ml of 2 N HCl. The next 3 ml of 2 N HCl were discarded and $^3$H 3-MT was eluted with 12 ml 3 N HCl. The eluates were dried and counted by liquid scintillation. The rate of accumulation was corrected for the specific activity of tyrosine in each animal.

The data were analyzed by one- and two-way ANOVA and Dunnett's *t* test [30].

## RESULTS

### *Experiment 1*

Simultaneous analysis of drug and strain effects on norepinephrine synthesis showed that cycloheximide significantly inhibited norepinephrine synthesis, $F(3,44)=9.87$, $p<0.001$, whereas mouse strain had no effect. Subsequent analysis within each strain showed that all doses of cycloheximide significantly inhibited norepinephrine synthesis in the C57 strain ($t>3.30$, $p<0.01$ in each case), whereas only the highest dose had a significant effect in the DBA strain ($t>2.3$, $p<0.05$; Table 2). Analysis of the effects of drug and strain on dopamine synthesis indicated that cycloheximide significantly inhibited dopamine synthesis, $F(3,45)=5.46$, $p<0.01$ and that there was a significant strain effect when drug-treated animals were compared, $F(1,31)=4.79$, $p<0.05$. Within strains, cycloheximide inhibited dopamine synthesis with each dose in the C57 strain ($t>2.3$, $p<0.05$) whereas none of the doses significantly affected synthesis in the DBA strain (Table 2). These drug- and strain-dependent effects could not be attributed to differences in the specific activity of tyrosine in the brain. As shown in Table 3, except for a significant increase with the 75 mg/kg dose in C57 mice ($t=3.06$, $p<0.01$), there were no significant differences in tyrosine specific activity among doses within each strain. In drug-treated mice, there were no strain differences with each dose level. To determine whether cycloheximide produces similar effects on cerebral protein synthesis, the degree of incorporation of $^3$H-tyrosine into acid-insoluble protein was estimated. Each dose of cycloheximide caused a significant inhibition of tyrosine incorporation ($t>4.2$, $p<0.001$) (Table 3). The percent inhibition of incorporation ranged from 87.0 (50 mg/kg) to 91.9 (100 mg/kg) in the C57 strain and from 88.2 (50 mg/kg) to 90.0 (100 mg/kg) in the DBA strain. There were no significant differences between strains at any dose level.

TABLE 1

DRUG TREATMENT SCHEDULE FOR EXPERIMENT 2

| Group | 60 min | 30 min |
|---|---|---|
| 1 | Saline | Saline |
| 2 | Saline | *d*-AMP |
| 3 | CXM | Saline |
| 4 | CXM | *d*-AMP |

Saline or cycloheximide (CXM, 100 mg/kg) was administered subcutaneously 60 min prior to sacrifice. Saline or *d*-amphetamine (*d*-AMP, 5 mg/kg) was administered intraperitoneally 30 min prior to sacrifice.

TABLE 2

EFFECT OF CYCLOHEXIMIDE ON BRAIN CATECHOLAMINE SYNTHESIS IN TWO MOUSE STRAINS

| | Norepinephrine | | Dopamine | |
|---|---|---|---|---|
| Dose (mg/kg) | C57BL/6J | DBA/2J | C57BL/6J | DBA/2J |
| 0 | 32 ± 5 (8) | 37 ± 8 (7) | 88 ± 18 (9) | 82 ± 19 (7) |
| 50 | 16 ± 2† (8) | 19 ± 7 (5) | 48 ± 7† (8) | 75 ± 14 (5) |
| 75 | 9 ± 1† (7) | 15 ± 5 (5) | 43 ± 7† (7) | 54 ± 10 (6) |
| 100 | 9 ± 2† (6) | 13 ± 3† (5) | 28 ± 7† (6) | 37 ± 9 (5) |

Animals were administered various doses of cycloheximide and sacrificed 60 min later. Numbers in parentheses refer to number of animals.

*ng/g/15 min ± 1 SE.

†Significantly different from control ($p<0.05$).

### *Experiment 2*

As had been shown in Experiment 1, cycloheximide, 100

TABLE 3

EFFECT OF CYCLOHEXIMIDE ON SPECIFIC ACTIVITY OF TYROSINE AND INCORPORATION OF $^3$H TYROSINE INTO CEREBRAL PROTEIN IN TWO MOUSE STRAINS

| Dose (mg/kg) | Tyrosine specific activity* C57BL/6J | Tyrosine specific activity* DBA/2J | Tyrosine incorporation ratio† C57BL/6J | Tyrosine incorporation ratio† DBA/2J |
|---|---|---|---|---|
| 0 | 28.6 ± 2.9 (9) | 43.6 ± 5.7 (7) | 0.98 ± .07 (9) | 1.08 ± .19 (7) |
| 50 | 35.8 ± 2.4 (8) | 36.7 ± 4.2 (5) | 0.13 ± .01‡ (8) | 0.13 ± .01‡ (5) |
| 75 | 42.8 ± 3.5‡ (6) | 39.3 ± 5.1 (6) | 0.10 ± .01‡ (6) | 0.11 ± .01‡ (6) |
| 100 | 34.7 ± 4.7 (6) | 36.0 ± 4.2 (5) | 0.08 ± .01‡ (6) | 0.11 ± .02‡ (5) |

Animals were administered various doses of cycloheximide and sacrificed 60 min later. Numbers in parentheses refer to number of animals.

*nCi/μg ± 1 SE.

†The incorporation ratio was calculated by dividing the DPMs of radioactivity in the perchloric acid precipitate by the DPMs in the tissue supernate.

‡Significantly different from control ($p<0.05$).

TABLE 4

EFFECT OF CYCLOHEXIMIDE AND *d*-AMPHETAMINE ON RATE OF FORMATION OF CATECHOLAMINES AND 0-METHYLATED METABOLITES IN C57BL/6J MICE

| Treatment | NE | DA | NM | 3-MT |
|---|---|---|---|---|
| Control | 38 ± 4* (5) | 52 ± 8 (8) | 30 ± 5 (8) | 37 ± 6 (8) |
| *d*-AMP | 40 ± 8 (6) | 54 ± 10 (8) | 51 ± 17 (8) | 48 ± 10 (8) |
| CXM | 22 ± 3‡ (6) | 28 ± 4‡ (8) | 30 ± 5 (8) | 43 ± 6 (8) |
| CXM + *d*-AMP | 29 ± 6 (5) | 43 ± 11 (9) | 51 ± 10 (8) | 52 ± 6 (8) |

Animals received drug injections according to the protocol given in Table 1. The rates of accumulation of $^3$H norepinephrine (NE), dopamine (DA), normetanephrine (NM) and 3-methoxytyramine (3-MT) were determined as described in Materials and Methods. Numbers in parentheses refer to number of animals.

*ng/g/15 min ± 1 SE.

‡Significantly different from control ($p<0.05$).

mg/kg, significantly decreased the synthesis of norepinephrine ($t=2.57$, $p<0.05$) and dopamine ($t=2.81$, $p<0.02$) in C57 mice (Table 4). The drug had no significant effects on the rate of formation of the two metabolites. Administration of *d*-amphetamine alone increased the concentration of labelled metabolites but this was not statistically significant. When *d*-amphetamine was administered to mice pretreated with cycloheximide, there was a partial reversal or attenuation of the inhibition of norepinephrine and dopamine synthesis caused by cycloheximide. There were no significant differences in norepinephrine and dopamine synthesis rates between control animals and those receiving both drugs. Confirming the results obtained in Experiment 1, this dose of cycloheximide also decreased brain catecholamine synthesis in the DBA strain although the differences were not statistically significant (Table 5). However, in contrast to the results obtained with C57 mice, administration of *d*-amphetamine to cycloheximide-pretreated mice produced a marked, significant decrease in the rate of accumulation of labelled norepinephrine ($t=3.92$, $p<0.005$), dopamine ($t=2.58$, $p<0.05$), and normetanephrine ($t=2.21$, $p<0.05$), when compared to control animals. Analysis of drug and strain effects in mice receiving cycloheximide and cycloheximide+*d*-amphetamine revealed a significant drug-strain interaction for normetanephrine, $F(1,27)=5.15$, $p<0.05$, and a nearly significant drug-strain interaction for norepinephrine, $F(1,21)=4.13$, $p<0.06$ and dopamine, $F(1,27)=4.08$, $p<0.06$. This suggests that the administration of *d*-amphetamine to cycloheximide-pretreated mice affected the two strains differently.

TABLE 5

EFFECTS OF CYCLOHEXIMIDE AND *d*-AMPHETAMINE ON RATE OF FORMATION OF CATECHOLAMINES AND 0-METHYLATED METABOLITES IN DBA/2J MICE

| Treatment | NE | DA | NM | 3-MT |
|---|---|---|---|---|
| Control | 55 ± 9* (8) | 105 ± 27 (7) | 51 ± 10 (8) | 56 ± 15 (7) |
| *d*-AMP | 39 ± 8 (8) | 73 ± 11 (8) | 45 ± 7 (8) | 71 ± 19 (8) |
| CXM | 32 ± 7 (8) | 55 ± 11 (8) | 42 ± 10 (8) | 59 ± 15 (7) |
| CXM + *d*-AMP | 15 ± 1‡ (6) | 28 ± 5‡ (6) | 27 ± 4‡ (7) | 42 ± 12 (8) |

Refer to legend of Table 3.

*ng/g/15 min ± 1 SE.

‡Significantly different from control ($p<0.05$).

## DISCUSSION

Cycloheximide has been shown to inhibit the synthesis of brain catecholamines [3,10] and the results of the present study suggest that the drug exerts this effect in varying degrees in the C57 and DBA strains, at least in regard to dopamine synthesis.

One important implication of this strain difference concerns the reported differences in these two strains of the amnesic effect of cycloheximide [22,28]. Possible explanations of this difference which have been suggested include differences in the time course of protein synthesis inhibition [16,22] or degree of inhibition of protein synthesis [11]. However, Day and coworkers [7] did not find a correlation between rates of recovery of cerebral protein synthesis and differences in learning behavior after treatment with cycloheximide. Although a dose-dependent effect of cycloheximide on amnesia has been observed in the DBA strain, this was not associated with a dose-related effect on the degree of protein synthesis inhibition [22]. This was further supported by the present study which indicated that 60 min after the injection of cycloheximide, which approximates the time interval used in most training studies, there were no significant differences in the inhibition of protein synthesis between the two strains.

The difference in the sensitivity of these two mouse strains to the inhibition of catecholamine synthesis caused by cycloheximide suggest an alternative mechanism which could be responsible for the difference in sensitivity to the amnesic effects, in that brain dopamine synthesis is impaired to a greater extent in the C57 strain one hour after the drug injection, i.e., during the time period when long-term memory is believed to be formed.

There is considerable evidence that brain catecholamines may have an important role in mechanisms which regulate the formation or retention of long-term memory. For example, inhibitors of catecholamine synthesis such as FLA-63, diethyldithiocarbamate and $\alpha$-methyltyrosine have been shown to disrupt memory processes [9,12]. Central administration of catecholamines [14,27], on the other hand, have been shown to enhance such processes.

Since several drugs which enhance central catecholamine neuron activity are capable of blocking or reversing the behavioral effects of cycloheximide [18–20], it might be expected that differences in biochemical effects might occur in these two strains following treatment with cycloheximide and *d*-amphetamine. Although the dose of cycloheximide used in this experiment tended to decrease the synthesis of norepinephrine and dopamine in both strains, the administration of *d*-amphetamine to mice pretreated with cycloheximide produced strikingly different effects. As has been reported previously [3] *d*-amphetamine partially prevented the decrease in norepinephrine and dopamine synthesis caused by cycloheximide in the C57 strain in the present study. However, in the DBA strain, the synthesis of catecholamines was lower after treatment with both drugs than after cycloheximide alone. This strain difference was also apparent in the rate of formation of normetanephrine, which may reflect the rate of release of norepinephrine [5]. Whereas amphetamine tended to increase the formation of normetanephrine in C57 mice pretreated with cycloheximide, the reverse was true in the DBA strain. It is possible that cycloheximide may have affected the uptake of *d*-amphetamine [1] and selectively altered the brain concentration of the drug in these two strains. A dose-dependent effect of *d*-amphetamine on catecholamine synthesis has been shown in mice [23]. Such an effect could account for the observed strain differences.

Most of the studies which have shown that cycloheximide-induced amnesia can be reversed by *d*-amphetamine have utilized mice of the C57BL/6J [18,20] or Swiss [2] strains. Although there have been no published studies indicating whether this interaction occurs in DBA/2J mice, the results of the present study suggest that *d*-amphetamine may not be as effective in this strain. Moreover, it is of interest that *d*-amphetamine did not alter the amnesic effects of reserpine in the DBA/2J strain [8].

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