Drug-Induced Hyperthermia and Amphetamine Toxicity

By HAROLD H. WOLF and DAVID J. GEORGE*

Experiments were conducted to determine the relative importance of hyperthermia as a factor influencing the toxicity of amphetamine in aggregated and isolated mice. The ability of several agents to interfere with the hyperthermic response to amphetamine was compared with their ability to alter the incidence of lethality to this drug. It was evident that there was no direct relationship between the ability of the selected drugs to prevent the body temperature of mice from reaching the maximum temperature level of amphetamine-treated controls and their ability to protect mice from amphetamine-induced lethality. It was concluded that hyperthermia does not, in itself, appear to be the causative factor for the increased incidence of mortality to amphetamine observed in aggregated *versus* isolated mice.

MORE THAN 20 years have elapsed since Gunn and Gurd (1) first observed that amphetamine was several times more lethal to aggregated than to isolated mice. In recent years this phenomenon, known as the amphetamine aggregation effect, has served as a research tool in the search for new psychotherapeutic agents (2, 3). Although many potentially useful ataractics have been shown to protect against this phenomenon, the means whereby these agents alter the lethality to amphetamine remains unclear. In part this is due to a lack of insight concerning the mechanism whereby a heightened degree of social contact markedly enhances the toxicity of this drug.

It has been demonstrated that the amphetamine aggregation effect can be influenced by a large number of complex variables, including cage size (4, 5), body weight and degree of hydration (5, 6), environmental noise (6), animal strain (6, 7), ambient temperature (4, 6, 8), and exposure to stress (9). Swinyard, *et al.* (10), have suggested that the increased lethality seen with aggregation is related to anxiety or fear induced by a sudden change in the environment of the animal. Askew (11) has reported that the obvious increase in body temperature observed after the administration of amphetamine is also a major contributory factor to the aggregation effect. These findings contrast sharply with the earlier observations of Swinyard, *et al.* (10), who reported that alterations in body temperature cannot be considered a critical factor in the enhanced toxicity exhibited by amphetamine in aggregated animals.

In view of the above it appeared of interest to investigate in detail the relative importance of hyperthermia in the expression of the amphetamine aggregation phenomenon. The results obtained constitute the basis for this report.

METHODS

The experimental animals employed were novice, adult, male, albino mice of a random bred Swiss strain (Research Animal Associates, Columbus, Ohio). These animals, which ranged in weight from 16 to 26 Gm., were housed in groups of 25 in opaque plastic cages 45 × 24 × 12 cm. with wire mesh tops. They were maintained on Purina laboratory chow and had free access to food and water, except during the experimental test periods.

All temperature studies were conducted in both an aggregated and isolated environment. Aggregation was defined as placing three mice in a metal cage 7 × 7 × 7.5 cm. with a wire mesh bottom; isolation consisted of placing a solitary mouse in a similar cage. The room temperature in all instances was maintained at 24–26°.

Throughout this entire study racemic amphetamine sulfate[1] was administered intraperitoneally in a constant dose of 100 mg./Kg. With mice of the above described strain, this dose of amphetamine is lethal to approximately 30% of isolated and 60% of aggregated animals. Thus any drug-induced increment or decrement in amphetamine lethality could be observed. The following drugs were evaluated for their ability to alter amphetamine-induced lethality and hyperthermia: chlorpromazine,[2] acetylsalicylic acid, and phenoxybenzamine.[3] These drugs were all administered intraperitoneally in aqueous solution, except for acetylsalicylic acid, which was given orally in a 1% methylcellulose suspension. The concentration of the drug solution or suspension employed was such that the dose administered always represented 1 ml. per 100 Gm. body weight. The mean neurotoxic dose (TD_{50}) was determined for each drug by employing a modification of the method of Dunham and Miya (12). The end point for minimal neurotoxicity was muscular incoordination based on the inability of the mouse to remain for 1 minute, given three

Received September 17, 1963, from the College of Pharmacy, The Ohio State University, Columbus.
Accepted for publication October 15, 1963.
This investigation was supported in part by research grants from the Ohio State University Development Fund and the National Science Foundation.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.
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[1] Marketed as Benzedrine Sulfate by Smith Kline and French.
[2] Marketed as Thorazine Hydrochloride by Smith Kline and French.
[3] Marketed as Dibenzyline Hydrochloride by Smith Kline and French.

successive trials, on a horizontal rod 2 cm. in diameter, rotating at 8 r.p.m., and suspended 38 cm. above a flat surface. Groups of at least nine mice were given various doses of drug until a minimum of three points were established in the range between 0 and 100% minimal neurotoxicity. Observations were made every 30 minutes up to a period of 8 hours. The results obtained were then plotted on logarithmic probability paper and a regression line was fitted to the plotted points by eye. From this plot of the data the TD_{50} and 95% fiducial limits were calculated by the method of Litchfield and Wilcoxon (13). The time of peak activity for each drug was determined by inspection of the neurotoxicity *versus* time data, and thereafter all test procedures were designed to coincide with this time. To insure that comparisons were made on the basis of equipotent doses, all drugs were administered in amounts equivalent to similar fractions of their respective TD_{50}'s.

To determine the effects of the agents employed on body temperature, *per se*, a series of experiments was conducted in which rectal temperatures were recorded immediately prior to drug administration and hourly thereafter for a period of 3 hours. The temperature was taken using a Tele-Thermometer and thermocouple probe (Yellow Springs Instrument Co.) inserted 2 cm. into the rectum for a period of 30 seconds. The number of mice in each drug treatment group ranged from 12 to 15, and saline controls were included for both the aggregated and isolated situations.

TABLE I.—TIME OF PEAK ACTIVITY AND NEUROTOXICITY OF SOME SELECTED DRUGS IN MICE[a]

Drug	Time Peak Effect[b]	TD_{50}[c]
Acetylsalicylic acid	90	682 (627–744)
Chlorpromazine	60	4.3 (3.1–5.9)
Phenoxybenzamine	180	50 (40–62)

[a] Values in parenthesis are 95% fiducial limits. [b] Minutes. [c] mg./Kg.

To test the hypothesis that drugs which protect against amphetamine-induced lethality do so by preventing body temperature from reaching a critical maximum level, groups of at least 24 animals each were first pretreated with varying doses of chlorpromazine, acetylsalicylic acid, or phenoxybenzamine and later challenged during the time of peak drug activity with 100 mg./Kg. of amphetamine. Rectal temperatures and the incidence of lethality were recorded hourly for 3 hours following amphetamine administration. As in previous studies, the experiments were conducted under conditions of both isolation and aggregation and controls pretreated with a requisite volume of saline were included. Any mice which died during the course of the experiment were not removed from the metal cages. All lethality data were statistically analyzed by means of a chi square test; temperature data were compared on the basis of means and standard errors.

RESULTS

The time of peak effect and neurotoxicity of the compounds in mice are shown in Table I. The time of peak effect measured by the test for minimal neurotoxicity varied from 1 hour for chlorpromazine to 3 hours for phenoxybenzamine. Acetylsalicylic acid exhibited peak activity at 90 minutes. Acetylsalicylic acid, chlorpromazine, and phenoxybenzamine induced overt symptoms of neurotoxicity in 50% of the animals after doses of 682, 4.3, and 50 mg./Kg., respectively. All future doses of these drugs were given in quantities amounting to 1/4, 1/2, and 1 TD_{50}.

The effects of the drugs employed on the body temperature of mice maintained in both an isolated and aggregated situation are shown in Table II. These results demonstrate the influence of each drug when administered alone on body temperature.

As may be observed from Table II, amphetamine, at a dose level of 100 mg./Kg., was markedly hyperthermic; the peak response occurring 2 hours

TABLE II.—INFLUENCE OF SOME SELECTED DRUGS ON RECTAL TEMPERATURE OF MICE

Environmental Condition	Drug	Dose[a]	Mice, No.	Hr. after Drug Administration 0	1	2	3
Isolation	Saline (control)	...	72	38.2[b]	37.6	37.4	37.1
	Amphetamine	[c]	99	38.0	39.0[d]	39.7[d]	39.3[d]
	Chlorpromazine	1/4	12	38.6	38.0	37.8	38.2
		1/2	12	38.6	37.3	36.3	37.0
		1	12	38.4	35.4[d]	32.1[d]	31.9[d]
	Acetylsalicylic acid	1/4	12	38.1	37.5	37.4	36.9
		1/2	12	38.3	36.9	36.9	37.0
		1	12	38.9	38.0	37.5	37.7
	Phenoxybenzamine	1/4	12	38.6	36.3	35.3[d]	34.8[d]
		1/2	15	37.2	35.0[d]	33.6[d]	33.1[d]
		1	12	38.3	35.1[d]	33.8[d]	33.8[d]
Aggregation	Saline (control)	...	72	38.2	37.2	37.2	37.4
	Amphetamine	[c]	98	37.8	39.4[d]	39.9[d]	39.8[d]
	Chlorpromazine	1/4	12	38.5	37.8	37.4	37.8
		1/2	12	38.5	37.1	36.6	36.8
		1	12	38.6	37.0	35.8[d]	35.4[d]
	Acetylsalicylic acid	1/4	12	38.2	37.7	37.4	37.2
		1/2	12	37.9	37.2	37.0	37.0
		1	12	38.8	38.0	37.8	38.0
	Phenoxybenzamine	1/4	12	37.6	35.2[d]	34.3[d]	34.0[d]
		1/2	15	38.3	36.5	35.4[d]	35.5[d]
		1	12	38.3	34.9[d]	34.6[d]	34.2[d]

[a] Fractions of TD_{50}. [b] Average rectal temperature in °C. [c] 100 mg./Kg. [d] Significantly different from control ($p < .05$).

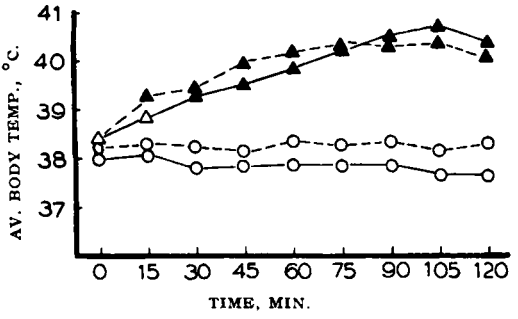

Fig. 1.—The influence of isolation and aggregation on amphetamine-induced hyperthermia. Key: O—O, isolated-saline (control); O- - -O, aggregated-saline (control); Δ—Δ, isolated-amphetamine, 100 mg./Kg.; Δ- - -Δ, aggregated-amphetamine, 100 mg./Kg.; and ▲, significantly different from control ($p < .05$).

after drug administration and amounting to 39.7° and 39.9° in the isolated and aggregated environments, respectively. It is also evident from Table II that, of the three agents under investigation, only phenoxybenzamine had a significant effect on body temperature at all dose levels employed. This agent demonstrated marked hypothermia under both environmental conditions, causing rectal temperature to fall to 33.1° 3 hours after administration of 1/2 TD_{50} followed by isolation and to 34.0° 3 hours after administration of 1/4 TD_{50} followed by aggregation. Chlorpromazine produced a hypothermic response only when given in doses equivalent to the full TD_{50}. Three hours after administration of this dose, body temperature fell to 31.9° and 35.4° in the isolated and aggregated environments, respectively. Acetylsalicylic acid in the dose levels employed appeared to have no appreciable effect on body temperature.

To explore more fully the hyperthermic response to amphetamine in an isolated *versus* aggregated environment, an experiment was carried out in which the rectal temperatures of 24 animals were recorded every 15 minutes for a 2-hour period following the administration of either 100 mg./Kg. of amphetamine or a requisite volume of saline (controls). The results of this study are shown in Fig. 1. As indicated in this figure, amphetamine was decidedly hyperthermic, causing body temperature to rise to a maximum of 40.7° in the isolated environment and 40.4° in the aggregated environment. It is also evident that this elevation in body temperature was quite rapid in its onset. However, it should be noted that essentially no difference in degree of hyperthermia was achieved in isolated *versus* aggregated animals.

The ability of the agents selected to alter amphetamine-induced hyperthermia and lethality can be observed in Table III. It is evident that the degree of hyperthermia achieved in the isolated saline-pretreated controls was not significantly different from that achieved by their aggregated counterparts. Pretreatment with chlorpromazine significantly interfered with amphetamine-induced hyperthermia when given to isolated animals in doses of 1/2 and 1 TD_{50}, whereas only the full TD_{50} was effective in the case of aggregation. On the other hand, pretreatment with acetylsalicylic acid (1/2 TD_{50}) altered amphetamine-induced hyperthermia in aggregated animals without significantly influencing the response in isolated mice. The rapid onset of lethality in animals which had been pretreated with 1/4 or 1 TD_{50} of acetylsalicylic acid and then subjected to amphetamine precludes an interpretation of temperature data for these dose levels. It is quite evident that, of the three agents evaluated, only phenoxybenzamine interfered with amphetamine-induced pyrexia in both environmental situations and at all dose levels employed.

The lethality data presented in Table III clearly demonstrate the existence of the amphetamine aggregation phenomenon in that 28.3% of the saline-pretreated control animals died in isolation, whereas 55.1% of a similarly treated group of animals succumbed to the combined effects of amphetamine and aggregation. Chlorpromazine may be observed to have decreased significantly the incidence of lethality to amphetamine in both environmental situations and at all dose levels used. On the other hand, acetylsalicylic acid afforded no protection against amphetamine-induced lethality and, indeed, seemed to potentiate the response. For example, it can be seen that when mice which had been pretreated with acetylsalicylic acid (1/4, 1/2, or 1 TD_{50}), followed by amphetamine were subjected to aggregation, the incidence of lethality rose to 100% in all cases. This same result was observed in isolated animals pretreated with the full TD_{50} of acetylsalicylic acid prior to the administration of amphetamine. Phenoxybenzamine appeared to be especially effective in abolishing the

TABLE III.—AVERAGE MAXIMUM TEMPERATURE AND LETHALITY OF MICE PRETREATED WITH SELECTED DRUGS AND ADMINISTERED AMPHETAMINE[a]

Drug	Dose Fractions of TD_{50}	Isolated Mice: Temp., °C.[b]	Isolated Mice: % Lethality[c]	Aggregated Mice: Temp., °C.[b]	Aggregated Mice: % Lethality[c]
Saline (control)	...	40.2 ± 0.8	28.3	40.6 ± 0.3	55.1
Chlorpromazine	1/4	39.8 ± 0.5	8.3[d]	40.2 ± 0.5	33.3[d]
	1/2	38.9 ± 0.4[d]	4.2[d]	40.2 ± 0.5	33.3[d]
	1	37.8 ± 0.3[d]	8.3[d]	38.9 ± 0.7[d]	30.6[d]
Acetylsalicylic acid	1/4	40.3 ± 0.6	33.3	[e]	100[d]
	1/2	40.1 ± 0.6	33.3	39.4 ± 0.7[d]	100[d]
	1	[e]	100[d]	[e]	100[d]
Phenoxybenzamine	1/4	36.5 ± 0.6[d]	4.2[d]	38.4 ± 0.4[d]	16.6[d]
	1/2	36.8 ± 0.8[d]	16.6	39.5 ± 0.4[d]	29.2[d]
	1	36.4 ± 1.1[d]	20.8	38.2 ± 0.7[d]	29.2[d]

[a] All mice received amphetamine, 100 mg./Kg. [b] Average of maximum rectal temperatures recorded during 3 hours. Values representing 95% confidence limits are included. [c] Three-hour observation period. [d] Significantly different from control ($p < .05$). [e] Lethality occurred before a significant number of rectal temperatures could be recorded.

amphetamine aggregation effect, in that all the dose levels employed in grouped mice significantly lowered the incidence of lethality to amphetamine. However, in an isolated situation, protection could only be achieved with a dose equivalent to $^1/_4$ TD_{50}.

DISCUSSION

The data presented indicate that the degree of hyperthermia induced by amphetamine cannot fully account for the increased incidence of lethality to this drug observed in aggregated *versus* isolated mice. This is evident from the results seen in Fig. 1 and Table III (saline-pretreated controls) which demonstrate that choice of environment did not significantly influence the hyperthermic response to amphetamine. The results also tend to refute the hypothesis that drugs which offer protection against the amphetamine aggregation phenomenon do so by means of a hypothermic mechanism. The information presented in Table II demonstrates that small doses of chlorpromazine, an agent well known to abolish the aggregation phenomenon (2, 3, 9, 10), were not hypothermic *per se* in this environmental situation.

Askew (11) has reported that the incidence of amphetamine-induced lethality in female Schofield albino mice can be predicted on the basis of body temperature. Mice whose body temperature exceeds a critical level (42.4°) will die, whereas those whose body temperature remains below a certain level (41.8°) will survive. In this same report, the author provides results which demonstrate that body temperature rises to a higher level in aggregated than in isolated mice, both receiving identical doses of amphetamine. On the basis of these data, the author postulates that a significant increase in body temperature in an aggregated *versus* isolated environment could account for the greater toxicity of amphetamine seen in the grouped mice. Recently Askew (14) has presented evidence to support the hypothesis that there is a direct relationship between the ability of drugs to reduce amphetamine-induced hyperthermia and their ability to protect against the amphetamine aggregation phenomenon. Table III presents a collection of data which do not support this hypothesis and Table IV summarizes these observations.

It is evident that there was no direct relationship between the ability of the selected drugs to prevent the body temperature of mice from reaching the maximum level of amphetamine-treated controls and their ability to protect mice from amphetamine-induced lethality. Chlorpromazine demonstrated protection in both isolated and aggregated mice at dose levels which had no significant effect on the degree of hyperthermia induced by amphetamine. Acetylsalicylic acid offered no protection at any dose employed in either the isolated or aggregated situation. Indeed, in all instances involving aggregation this agent was observed to increase the incidence of lethality. It is important to note that this occurred despite the observation that acetylsalicylic acid ($^1/_2$ TD_{50}) did significantly interfere with the hyperthermic response to amphetamine. Furthermore, it can be seen that phenoxybenzamine, which had been noted to interfere markedly with amphetamine-induced hyperthermia in both environmental situations, offered consistent protection against the lethal effects of the drug only in the aggregated environment. Thus, almost every combination of protection and temperature effect was observed.

There are several possible reasons for the discrepancy in results reported by Askew (14) and those presented above. The experimental methods employed in the two studies differed considerably with respect to parameters which have previously been demonstrated to influence markedly the amphetamine aggregation phenomenon, *e.g.*, cage size, degree of aggregation, drug dosage levels, and animal strain. Moreover, our experiments were terminated at the end of 3 hours, whereas Askew's were carried out over intervals of 6 and 7 hours; thus the results obtained may reflect "acute" *versus* "long term" effects of the drugs. It is also possible that the large amount of animal manipulation employed in Askew's procedure (rectal temperatures were taken every 20 minutes up to 2 hours and then every hour until termination of the experiment) may have contributed to the results reported.

It should be emphasized that since the work described herein was essentially complete at the time of Askew's recent publication (14), and since the experimental conditions were by no means analogous in the two situations, the results presented are not to be interpreted as an unsuccessful attempt to replicate that author's work. On the other hand, on the basis of our observations it would appear that, while amphetamine is indeed hyperthermic, a causal relationship does not always exist between this hyperthermia and the mechanism whereby an alteration in social environment can markedly increase the

TABLE IV.—SUMMARY OF EFFECTS OF SELECTED DRUGS ON AMPHETAMINE-INDUCED[a] LETHALITY AND HYPERTHERMIA

Drug	Dose[b]	Isolated Mice: Diminished Lethality[c]	Isolated Mice: Diminished Hyperthermia[d]	Aggregated Mice: Diminished Lethality[c]	Aggregated Mice: Diminished Hyperthermia[d]
Chlorpromazine	1/4	Yes	No	Yes	No
	1/2	Yes	Yes	Yes	No
	1	Yes	Yes	Yes	Yes
Acetylsalicylic acid	1/4	No	No	No	[e]
	1/2	No	No	No	Yes
	1	No	[e]	No	[e]
Phenoxybenzamine	1/4	Yes	Yes	Yes	Yes
	1/2	No	Yes	Yes	Yes
	1	No	Yes	Yes	Yes

[a] All mice received amphetamine, 100 mg./Kg. [b] Fractions of TD_{50}. [c] Significantly decreased amphetamine-induced lethality ($p < .05$). [d] Significantly interfered with amphetamine-induced hyperthermia ($p < .05$). [e] Data not available.

toxicity of this drug. Moreover, in view of two recent reports which indicate that magnitude of prior social experience (15) and quantity of body contact (16) represent important factors influencing the expression of the amphetamine aggregation effect, it is evident that this phenomenon represents the product of a complex interaction between several environmental, genetic, and pharmacological factors.

SUMMARY

A series of experiments was designed to test the hypothesis that hyperthermia is a major factor contributing to the increased toxicity of amphetamine observed in aggregated *versus* isolated mice. Incidence of lethality and rectal temperatures were recorded hourly over a 3-hour interval following the intraperitoneal administration of 100 mg./Kg. amphetamine to animals placed in either an aggregated (3 mice per 7 × 7 × 7.5 cm. cage) or isolated environment. The effect of pretreatment with several agents known to influence body temperature (chlorpromazine, acetylsalicylic acid, and phenoxybenzamine) was also determined. On the basis of the results obtained the following conclusions appear to be justified:

Although amphetamine is definitely more toxic in grouped animals (aggregation effect), the degree of hyperthermia induced may be essentially the same for both aggregated (40.6°) and isolated (40.2°) mice.

Drugs which have the ability to protect animals against the occurrence of the amphetamine aggregation phenomenon, *e.g.*, chlorpromazine, may do so at dose levels which are not hypothermic *per se* and which do not alter amphetamine-induced hyperthermia.

Phenoxybenzamine, which has the ability to depress markedly the hyperthermic response to amphetamine, does not consistently abolish the lethality of this drug in an isolated environment.

Pretreatment with acetylsalicylic acid using a dose level which decreases the magnitude of amphetamine-induced hyperthermia significantly increases its lethality.

A generalization indicating a causal relationship between hyperthermia and the amphetamine aggregation phenomenon may be unwarranted.

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Spectrophotometric Method for the Assay of Individual Nitroglycerin Tablets

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A spectrophotometric procedure for the microdetermination of glyceryl trinitrate through alkaline hydrolysis and subsequent determination of the nitrite formed through diazotization and coupling has been devised which avoids the serious interference in the presence of lactose. The principal feature of the method is based on the substitution of strontium hydroxide for the usual sodium hydroxide as the hydrolyzing alkali. Presumably the formation of the strontium salt of lactose removes this carbohydrate from the field of interference. The results of more than 500 assays of individual tablets of nitroglycerin representing four different sources of manufacture and five different dosages are reported.

PROBABLY BECAUSE of its high degree of sensitivity and relative simplicity, a preferred method for the microdetermination of glyceryl trinitrate is the one based on the alkaline hydrolysis (1) of the nitrate ester and determination of the nitrite formed through the customary diazotization and coupling reaction and subsequent spectrophotometric measurement of the dye formed. An obvious application of this procedure would be the assay of individual nitroglycerin tablets in which suitable aliquots of a simple aqueous solution of the tablet would be subjected to the procedure.

However, it is known that lactose, which appears to be the excipient of preference in the manufacture of these tablets and is present in gross excess, seriously interferes with this analytical procedure (2). In a consideration of possible methods of avoiding this source of inter-

Received July 22, 1963, from the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore.
Accepted for publication October 8, 1963.