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Amphetamine toxicity and endogenous noradrenaline concentrations in isolated and aggregated mice

There is indirect evidence for the participation of noradrenaline in the mechanism by which aggregation augments amphetamine toxicity in mice. Experiments with amphetamine toxicity in mice pretreated with a variety of pharmacological agents known to modify the metabolism, storage, or action of noradrenaline indicate that noradrenaline released from endogenous stores plays a role in the aggregation effect (Sethy & Sheth, 1968). Although reduced tissue concentrations of noradrenaline in mice treated with amphetamine are consistent with this view (Moore, 1963, 1964; Beauvallet & Solier, 1964; Lal & Chessick, 1964; Menon & Dandiya, 1967), the relation between the enhanced toxicity of amphetamine in aggregated mice and noradrenaline depletion is not clear. We aimed to examine this relation by determining whether amphetamine-induced lethality, in either isolated or aggregated mice, is correlated with the degree of noradrenaline depletion in tissues. This approach differs from that used earlier (Moore, 1963, 1964; Lal & Chessick, 1964) in one important aspect. A distinction has been made between the degree of noradrenaline depletion in mice that died as a consequence of amphetamine treatment and in mice that survived the treatment.

Novice, male, albino mice of a random-bred Swiss strain (Maxfield; Cincinnati, Ohio), 9 to 12 weeks, 25 to 35 g, were housed 15 per cage (45 × 24 × 12 cm) for not less than 30 days; Purina laboratory chow and water were freely available. After an intraperitoneal injection of saline or (+)-amphetamine sulphate (30 or 100 mg/kg) in aqueous solution (1 ml/100 g), mice were either isolated or aggregated (3 per cage) in metal cages (7 × 7 × 7·5 cm) with a wire mesh side for observation. The rationale for using these doses of (+)-amphetamine has been previously discussed (George & Wolf, 1966, 1967). Ambient temperature was 24 ± 1°. After 3 h, surviving animals (survivors) were killed by cervical dislocation; their brains and hearts were removed and frozen in liquid nitrogen within 1 min after death. Mice that died within 3 h (non-survivors) had their tissues removed and frozen immediately after death. The degree of aggregation was maintained constant by replacing non-survivors with untreated mice. Noradrenaline in pooled samples of 4 hearts or 3 whole brains was assayed fluorometrically by the trihydroxyindole method of Anton & Sayre (1962).

Aggregation itself did not deplete brain or heart stores of noradrenaline (Table 1). The levels were not significantly different ($P>0{\cdot}05$) in saline-treated isolated versus aggregated mice. Furthermore, aggregation did not enhance the noradrenaline-depleting effect of (+)-amphetamine. Brain and heart noradrenaline levels in mice treated with 30 or 100 mg/kg of (+)-amphetamine were not significantly different in isolated versus aggregated animals. This finding differs from that of Lal & Chessick (1964) who found lower brain levels of noradrenaline in aggregated than in isolated mice 30 min after (+)-amphetamine (25 mg/kg). It is possible that high doses of (+)-amphetamine or long treatment times, such as we used, obscured any influence aggregation might have had on the noradrenaline-depleting effect of (+)-amphetamine. Moore (1963), too, reported that aggregation in mice enhanced noradrenaline deple-

Table 1. *Effect of (+)-amphetamine on noradrenaline concentrations in brains and hearts of isolated and aggregated mice.* Determinations were made on pooled organs (3 brains, 4 hearts) from mice that died within 3 h (non-survivors) or were killed 3 h (survivors) after treatment. Noradrenaline content expressed as mean values in $\mu g/g \pm$ s.e.; number of determinations is in parentheses.

Treatment	Lethality %	Noradrenaline* Brain		Heart	
		Non-survivors	Survivors	Non-survivors	Survivors
Saline					
Isolated	0	—	0·18 ± 0·01 (16)	—	0·46 ± 0·03 (11)
Aggregated	0	—	0·20 ± 0·01 (18)	—	0·48 ± 0·02 (13)
(+)-Amphetamine 30 mg/kg					
Isolated	7	†	0·08 ± 0·01 (8)	†	0·32 ± 0·03 (7)
Aggregated	52	0·04 ± 0·00 (10)	0·06 ± 0·01 (8)	0·10 ± 0·01 (7)	0·25 ± 0·03 (6)
+)-Amphetamine 100 mg/kg					
Isolated	31	0·13 ± 0·02 (7)	0·02 ± 0·00 (12)	0·22 ± 0·03 (5)	0·16 ± 0·02 (9)
Aggregated	67	0·13 ± 0·01 (10)	0·02 ± 0·00 (6)	0·24 ± 0·02 (6)	0·12 ± 0·02 (4)

* Noradrenaline concn in survivors or non-survivors within each treatment group are not significantly different ($P > 0·05$) in isolated versus aggregated mice. The largest difference shown (heart, survivors, 30 mg/kg dose) has $P = 0·22$.
† Low incidence of lethality did not permit determination of this value.

tion by (+)-amphetamine when brain and heart noradrenaline concentrations were measured 4 h after doses up to 40 mg/kg (+)-amphetamine. But while Lal & Chessick reported no deaths during their relatively short treatment period, there were many deaths during Moore's experiments. However, Moore made no distinction between noradrenaline depletion in survivors and non-survivors, and apparently combined the data from the two groups. In view of the differences we observed in noradrenaline levels in survivors and non-survivors, such a combination would yield misleading results.

The results from animals treated with 100 mg/kg of (+)-amphetamine show that death apparently is not due to a reduction of brain or heart noradrenaline below survival level. After this dose of (+)-amphetamine, noradrenaline concentrations in non-survivors were higher than those in survivors. Thus we find no obvious connection to exist between lethality and noradrenaline depletion in either brain or heart; differences in the degree of noradrenaline depletion may simply reflect the influence of dose and duration of action of (+)-amphetamine. Noradrenaline concentrations in survivors of the 100 mg/kg dose were lower than those observed in survivors of the 30 mg/kg dose, indicating that the depleting effect of (+)-amphetamine is dose-dependent. If the non-survivor and survivor data had been pooled, this dose-dependent relation would have been obscured. Almost all deaths (94%), regardless of the environmental situation, resulting from a dose of 100 mg/kg occurred within 60 min, whereas after 30 mg/kg, 97% of deaths occurred between 90 and 180 min after drug administration. The limited duration of action of (+)-amphetamine could explain why less depletion was seen in non-survivors treated with a dose of 100 mg/kg than in non-survivors that received the 30 mg/kg dose. Similarly, the shorter time available for depletion in non-survivors versus survivors at the 100 mg/kg dose (60 versus 180 min) might explain the higher noradrenaline concentrations observed in non-survivors.

These results suggest that the degree of depletion of endogenous stores of noradrenaline is not causally related to (+)-amphetamine lethality in isolated mice or the enhanced lethality in aggregated mice.

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Effects of mescaline and 2,5-dimethoxy-4-methylphenethylamine on sleeping time in mice

Mescaline shortens while 2,5-dimethoxy-4-methylphenethylamine (DMM-PEA) potentiates the sleeping time of pentobarbitone in mice (Ho, McIsaac & others, 1970; Ho, Tansey & others, 1970). We have now enquired whether the effect is explicable by an alteration in the metabolism of pentobarbitone.

Male Yale-Swiss mice, 25–30 g, were injected intraperitoneally with 50 μmol/kg of mescaline or DMM-PEA in saline. Control animals were given only saline. After 5 min a mixture of 40 mg/kg of sodium pentobarbitone and 100 μCi of [^{14}C]labelled pentobarbitone (New England Nuclear, U.S.A.) in saline was administered by the same route. The animals, groups of eight, were killed 30 and 60 min after pentobarbitone. The tissues from two mice of the same interval were combined and homogenized in three parts of water. Blood samples from a pool of two mice were centrifuged to separate the plasma. Tissue homogenates (0·1 ml) or plasma (25 μl) were treated with methanol and liquifluor, and assayed for ^{14}C by liquid scintillation. All values were corrected for 100% efficiency (channel ratio) and recovery. For chromatography, plasma or brain homogenate was extracted with diethyl ether (Cooper & Brodie, 1957). Sequential sections of paper (1 × 2·5 cm^2) from paper chromatograms were placed in counting vials, treated with methanol and liquifluor, and then assayed for ^{14}C. Sleeping time was recorded as the time between loss and return of the righting reflex after intraperitoneal injection of sodium barbitone (250 mg/kg) to mice.

At 60 min after intraperitoneal injection of [^{14}C]pentobarbitone, significant increases of specific activity were observed in the plasma and brain of animals pretreated with mescaline or DMM-PEA (Table 1). The two compounds also caused increases of radioactivity in liver at both 30 and 60 min intervals. The kidney concentration of pentobarbitone plus metabolites was higher than in the controls at 30 min, but decreased to the same level at 60 min.

From the chromatographic studies, the proportions of unchanged pentobarbitone and metabolites in plasma and brain of both experimental and control animals were calculated (Table 2). The recoveries of metabolites by ether extraction from the plasma and brain homogenates were 90 and 96% respectively. Decreases in the amounts of metabolites and increases in the unchanged pentobarbitone were observed in the 30 min plasma and 60 min brain samples of animals treated with DMM-PEA;