Hyperthermia and the amphetamine aggregation phenomenon: absence of a causal relation

That aggregation augments amphetamine toxicity in mice was first reported by Gunn & Gurd (1940) and the effect has been widely studied since Chance (1946) enumerated factors influencing this toxicity (see reviews by Thiesson, 1964; Sethy & Sheth, 1968). It has been reported that in amphetamine-treated, aggregated mice there is a critical rectal temperature below which all survive and above which lethality increases progressively (Askew, 1962). From this it has been inferred that the degree of hyperthermia is causally related to amphetamine-aggregation toxicity (Askew, 1962; Scott, Lee & Ho, 1971; Craig & Kupferberg, 1972). This contrasts with the report of Swinyard, Clark & others (1961) that alteration in body temperature cannot be considered a critical factor in the enhanced toxicity of amphetamine in aggregated mice, and with the observations of Wolf & George (1964) who found no direct relation between the ability of certain drugs to prevent amphetamine hyperthermia in mice and their ability to protect the animals from amphetamine-induced lethality. Because of the indirect evidence implicating catecholamines in the mechanism by which aggregation augments amphetamine toxicity (reviewed by Sethy & Sheth, 1968), and in the hyperthermic response to systemically administered amphetamine (Matsumoto & Griffin, 1971; Hill & Horita, 1971; Maling, Williams & Koppanyi, 1972), we have re-examined the relation between amphetamine-induced hyperthermia and group toxicity using mice subjected to peripheral sympathectomy. As (+) and (−)-amphetamine differ quantitatively in their ability to modify behaviour and catecholamine uptake (Taylor & Snyder, 1970) and thermoregulation (Maling & others, 1972), both isomers were used.

Novice, male albino mice (CF #1 strain; Carworth Farms, Michigan), 18–25 g were housed 20 per cage (45 × 24 × 12 cm) for 5–10 days; Purina laboratory chow and water were freely available. After an intraperitoneal injection of saline, (+)-amphetamine sulphate (30 mg kg^{-1}) or (−)-amphetamine sulphate (150 mg kg^{-1}) in aqueous solution (1 ml per 100 g), mice were either isolated or aggregated (3 per cage) in metal cages (7 × 7 × 7·5 cm). The choice of dose of (+)-amphetamine has been discussed (George & Wolf, 1966, 1967). While that of (−)-amphetamine was that which preliminary results had shown to be equally toxic with the (+)-isomer. Aggregated mice that died during the 3 h observation period were replaced by untreated mice to maintain aggregation. Body temperature was recorded every 15 min using a telethermometer and thermocouple probe (Yellow Springs Instrument Co.), inserted 2 cm into the rectum for 15s. Ambient temperature was 23·5 ± 1°. To produce peripheral sympathectomy, mice were given a single intravenous injection of 100 mg kg^{-1}, 6-hydroxydopamine hydrobromide in normal saline. Amphetamine toxicity was examined 48 h later. Time *vs* body temperature curves were constructed and 3 h mortality figures were subjected to χ^2 analyses (Siegel, 1956).

Essentially equivalent results for both isomers were obtained with the doses chosen (Table 1). Thus, in agreement with Moore (1963) both isomers are significantly more toxic in grouped *vs* isolated mice. It is also evident that 6-hydroxydopamine, in a dose severely affecting sympathetic nerve function (Smookler & Clarke, 1972), effectively reduced the toxicity produced by either isomer and virtually abolished the aggregation phenomenon. Since systemically administered 6-hydroxydopamine is known to produce an acute and selective degeneration of sympathetic adrenergic nerve terminals (Thoenen & Tranzer, 1968) without producing an effect on the whole brain concentration of noradrenaline (Clark, Carrodi & Masuoka, 1971), our results support the concept that peripheral release of catecholamines plays an important role in amphetamine aggregation toxicity and are in agreement with the observation that both

Table 1. *Effect of 6-hydroxydopamine (6-OHDA)* on amphetamine-induced lethality in mice.*

Drug	Dose mg kg^{-1}	Pre-treatment	Condition: Aggregated	Condition: Isolated	χ^2	P
(+)-Amphetamine	30	Saline	38/48 (79%)**	13/47 (27%)	23·3	<0·001†
(+)-Amphetamine	30	6-OHDA	4/30 (13%)	0/28 (0%)	2·3	n.s.
(—)-Amphetamine	150	Saline	43/57 (75%)	14/59 (23%)	28·9	<0·001
(—)-Amphetamine	150	6-OHDA	1/30 (3%)	0/30 (0%)	0·2	n.s.

* 100 mg kg^{-1} i.v.; administered 48 h before amphetamine.
** % dead in 3 h.
† Aggregated *vs* isolated comparison.

isomers of amphetamine stimulate adrenoceptors indirectly by releasing endogeneous catecholamines (Wolf, Rollins & others, 1969).

The results in Fig. 1a and b, show that both isomers can produce hyperthermia. However, there were no significant differences in the size of this response produced by either isomer in isolated aggregated animals. This does not support the concept that amphetamine-induced lethality is dependent upon body temperature exceeding a critical threshold (Askew, 1962). Moreover, (—)-amphetamine produced marked hypothermia in both isolated and aggregated mice before elevating body temperature (Fig. 1b).

In agreement with Simmonds & Uretsky (1970), 6-hydroxydopamine did not significantly alter thermoregulatory responses at room temperature (Fig. 1c and d), and while it abolished the aggregation phenomenon, it did not prevent the hyperthermic response produced by either amphetamine isomer (Fig. 1c and d). However, it did abolish the hypothermia produced by the (—)-isomer in aggregated animals (Fig. 1d). These results clearly indicate that with the strain of mice used the hyperthermic effects of the amphetamine isomers can be separated from their ability to produce enhanced toxicity in an aggregated environment.

Finally, in (+)-amphetamine-treated animals, the number of deaths that occurred

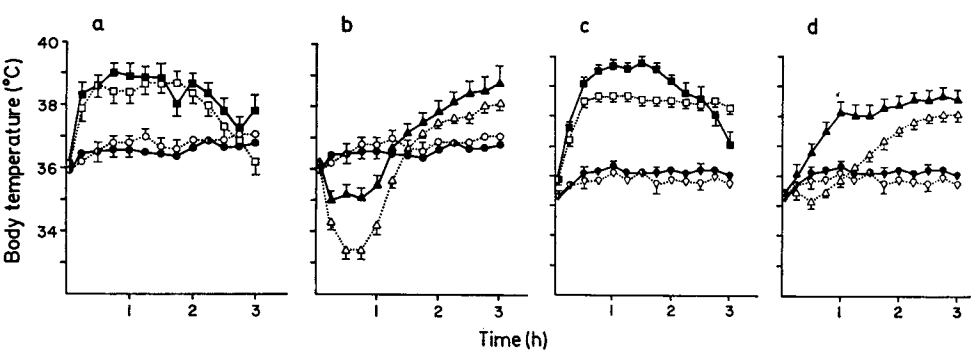

FIG. 1 (a). Effect of (+)-amphetamine (30 mg kg^{-1}) on body temperature in isolated (□) and aggregated (■) mice.
(b). Effect of (—)-amphetamine (150 mg kg^{-1}) on body temperature in isolated (△) and aggregated (▲) mice. Control animals received 0·9% saline and were isolated (○) or aggregated (●).
(c). Effect of (+)-amphetamine (30 mg kg^{-1}) on body temperature in isolated (□) and aggregated (■) mice pretreated with 6-hydroxydopamine (6-OHDA).
(d). Effect of (—)-amphetamine (150 mg kg^{-1}) on body temperature in isolated (△) and aggregated (▲) mice pretreated with 6-OHDA. Amphetamine was administered 48 h after a single i.v. injection of 100 mg kg^{-1} 6-OHDA. Control animals received 0·9% saline following similar 6-OHDA pretreatment and were isolated (▽) or aggregated (▼).
Vertical bracketed lines represent s.e. wherever this is greater than the plotting symbols used.

within 2 h (46%) was similar to that during the last hour (54%). In contrast, all (−)-amphetamine-induced lethality (100%) occurred within 2 h of drug administration, at a time when hypothermia was predominant (Fig. 1b). This again suggests that the aggregation phenomenon produced by amphetamine isomers is not causally related to their ability to induce hyperthermia.

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