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Mechanism of the protective effect of reserpine on aggregated mice treated with (+)-amphetamine

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Experiments were made to see if the effect of reserpine in protecting aggregated mice from the toxic effect of (+)-amphetamine depended on the hypothermia or on the depletion of brain noradrenaline it induces. In aggregated mice, a 7 mg/kg dose of amphetamine elevated body temperature, lowered the level of brain noradrenaline and caused 100% mortality. In reserpinized aggregated mice, amphetamine did not cause hyperthermia or any further depletion of brain noradrenaline. Prevention of the hypothermic effect of reserpine by keeping amphetamine-treated reserpinized animals at a higher environmental temperature markedly lowered the protective effect of reserpine. When also the depletion of noradrenaline by reserpine was antagonized by dopa, reserpine no longer protected aggregated mice from the toxic effect of amphetamine. The lowering of 5-hydroxytryptamine content brought about by reserpine remained unaltered during these procedures. Complete protection against amphetamine toxicity was also offered by α-methyl-1-tyrosine in doses which lowered brain noradrenaline to almost the same extent as reserpine, but which did not lower temperature or brain 5-hydroxytryptamine. When the body temperature of aggregated mice with brain noradrenaline lowered by α-methyl-1-tyrosine was elevated by subjecting the animals to heat stress, the protective effect was reduced. Hypothermia induced by reserpine could thus be related to its noradrenaline-depleting action. The results show that both properties contribute to reserpine's protective action. However, the abolition by dopa of this protective effect of reserpine and the complete protection offered by α-methyl-1-tyrosine without hypothermia suggest that depletion of brain noradrenaline plays the more important role in the protective effect of reserpine.

SINCE Chance (1947) reported that the toxicity of amphetamine is markedly enhanced in aggregated mice, the increased mortality has been attributed to enhanced motor activity and excitement (Greenblatt & Osterberg, 1961), hyperthermia (Greenblatt & Osterberg, 1961; Askew, 1962; Fink & Larson, 1962), excessive noradrenaline liberation (Maxwell, 1959; Weiss, Laties & Blanton, 1961; Moore, 1963) and enhanced brain excitability (Swinyard, Clarke & others, 1961).

The importance of hyperthermia as a contributing factor has been stressed by Askew (1962) and by Hardinge & Peterson (1963, 1964), although Wolf & George (1964) did not find this to be so. Amphetamine depletes brain noradrenaline (McLean & McCartney, 1961; Sanan & Vogt, 1962) and this is enhanced in aggregated mice (Moore, 1963). Since adrenergic blocking agents protect aggregated mice from the toxic effect of amphetamine (Maxwell, 1959; Weiss & others, 1961), excessive noradrenaline release by amphetamine could be considered a factor in the mechanism of amphetamine toxicity in aggregated mice.

Reserpine protects aggregated mice from the lethal effect of amphetamine (Burn & Hobbs, 1958). We have evaluated the relative importance of the two actions of reserpine, which could be interrelated, namely, hypothermia (Lessin & Parkes, 1957) and brain noradrenaline depletion (Holzbauer & Vogt, 1956) in reducing the toxicity in aggregated mice. The role of hypothermia was assessed in amphetamine-treated reserpinized mice kept at a higher environmental temperature to counteract the

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hypothermic action of reserpine without altering its noradrenaline-depleting activity on the brain. Experiments were also made in the reserpine treated mice in which both the hypothermic and the noradrenaline lowering effect of the tranquillizer were antagonized by DL-3,4-dihydroxyphenylalanine (dopa). Since reserpine is known to liberate 5-hydroxytryptamine (5-HT) as well as noradrenaline and the tranquillizing effect has been correlated with the change in 5-HT (Brodie, Tomich & others, 1957) brain 5-HT levels were measured in the brains of these animals. α-Methyl-1-tyrosine, the tyrosine hydroxylase inhibitor (Nagatsu, Levitt & Udenfriend, 1964) which causes a specific lowering of brain noradrenaline without affecting brain 5-HT (Spector, Sjoerdsma & Udenfriend, 1965) was also used.

Experimental

METHODS

Reserpine (Serpasil, Ciba), α-methyl-1-tyrosine (Merck, Sharp & Dohme) DL-dihydroxyphenylalanine (Nutritional Biochemical Corporation) and (+)-amphetamine sulphate (SKF), doses of which refer to the salt, were used. The solution of α-methyl-1-tyrosine was prepared according to Spector & others (1965). Reserpine was dissolved in a few drops of glacial acetic acid and the volume made up with distilled water. Other drugs were dissolved in distilled water. All the injections were made intraperitoneally and the volume of injected solutions was 0·01 ml/g body weight. Controls in which the animals were treated with the particular solvent were run simultaneously.

Albino mice (CDRI strain) were kept in identical conditions for a week before the experiments. These were made at a room temperature of $29 \pm 2°$. Aggregated mice (groups of 10) were kept in covered metallic cages (25 cm × 13 cm × 12 cm). Animals to be kept at an elevated temperature were put in a well aerated chamber at $38{\cdot}5 \pm 0{\cdot}5°$ immediately after amphetamine treatment. The same groups of animals were used to study changes in mortality rate and for determining the rectal temperature. Noradrenaline determinations were made in different groups of aggregated mice.

Influence of drugs and other procedures on the lethal effect of amphetamine in aggregated mice. Immediately after treating groups of animals with doses of amphetamine ranging from 3 to 10 mg/kg, the mice were aggregated and the number of animals dying hourly during the first 4 hr was determined. A dose of 7 mg/kg was lethal to all the mice and 6 mg/kg was lethal only to 50%. The 7 mg dose was used in experiments assessing protective effects and a 4 mg dose in those procedures where marked potentiation of amphetamine toxicity had to be demonstrated. This dose was lethal to 20% of the control animals.

Experimental procedures are described in Table 1. Immediately after amphetamine treatment, the animals were aggregated and were observed hourly for 4 hr. The mice not treated with amphetamine (groups I, III, V, VII, X and XII), were kept together in large cages, one for each

TABLE 1. PROCEDURES USED AND RESULTS OBTAINED IN THE EXAMINATION OF THE MODIFICATION OF AMPHETAMINE TOXICITY IN GROUPS OF 10 AGGREGATED MICE. Rectal temperatures were taken 18 hr after reserpine or its controls or 4 hr after the last dose of α-methyl-1-tyrosine, or its control, and measurements repeated at hourly intervals for 4 hr. Animals were killed for the estimation of amines 20 hr after reserpine, or its controls, or 6 hr after the last dose of α-methyl-1-tyrosine or its control. Heat-stressed groups (H.S.) were subjected to the elevated temperature (38·5°) 18 hr after reserpine, or its controls, or 20 hr after α-methyl-1-tyrosine or its control.

Group No.	Pretreatment	Amphetamine (7 mg/kg)	Animals dead within 4 hr/ Total No. of animals employed	No. animals	Whole brain content of noradrenaline 5-HT (μg/g wet tissue)	
I	Solvent of reserpine	Not given	0/10	6	0·401 ± 0·06	0·418 ± 0·03
II	Solvent of reserpine ..	18 hr later	10/10	9	0·313 ± 0·044 ($P < 0·05$) when compared with group I	0·410 ± 0·03
III	Reserpine[1] ..	Not given		6	0·148 ± 0·016 ($P < 0·001$) when compared with group II	0·108 ± 0·009 ($P < 0·001$) when compared with group II
IV	Reserpine ..	18 hr later	0/10 ($P < 0·001$) when compared with group II	6	0·152 ± 0·011	0·119 ± 0·008
V	Reserpine ..	Not given (H.S.)		6	0·149 ± 0·009	0·113 ± 0·01
VI	Reserpine ..	18 hr later (H.S.)	11/20 ($P < 0·001$) when compared with group IV	6	0·139 ± 0·011	0·113 ± 0·01
VII	Reserpine + dopa[2] ..	Not given		6	0·291 ± 0·028 ($P < 0·01$) when compared with group III	0·099 ± 0·02
VIII	Reserpine + dopa[2] ..	15 min after dopa	20/20 ($P < 0·001$) when compared with group IV	6	0·278 ± 0·019	0·141 ± 0·002
IX	Solvent of α-methyl-1-tyrosine ..	4 hr after the last dose	10/10	3	0·308 ± 0·04 ($P < 0·05$) when compared with group I	0·401 ± 0·01
X	α-Methyl-1-tyrosine[3] ..	Not given		6	0·150 ± 0·01 ($P < 0·001$) when compared with group I	0·389 ± 0·04
XI	α-Methyl-1-tyrosine[3] ..	4 hr after the last dose	0/20 ($P < 0·001$) when compared with those given only amphetamine 7 mg/kg	6	0·159 ± 0·008	0·400 ± 0·03
XII	α-Methyl-1-tyrosine[3] ..	Not given (H.S.)		6	0·149 ± 0·02	0·398 ± 0·01
XIII	α-Methyl-1-tyrosine[3] ..	4 hr after the last dose (H.S.)	7/20 ($P < 0·02$) when compared to group XI	6	0·148 ± 0·017	0·410 ± 0·04

[1] Dose of reserpine was 1 mg/kg in all these experiments.
[2] Dopa was administered in a dose of 200 mg/kg 18 hr after reserpine.
[3] Dose of α-methyl-1-tyrosine, 3 doses of 80 mg/kg each over 24 hr.

treatment, and were aggregated into groups of ten the day after drug or solvent administration and after taking the rectal temperature.

Rectal temperature of mice. Rectal temperature was taken hourly (Table 1) for 4 hr by introducing a probe 1·5 cm into the rectum.

Influence of various procedures on the noradrenaline and 5-HT *contents of whole brain of mice.* The noradrenaline content of whole brain was extracted and estimated at the appropriate time (Table 1) according to Shore & Olin (1958) and 5-HT according to Mead & Finger (1961). Deaths were highest during the second hour after amphetamine treatment,

so amine estimations were made during this hour. Animals already dead were not used for neurohormonal estimations.

Results

Influence on the mortality of aggregated mice treated with amphetamine. Pretreatment with reserpine (1 mg/kg) offered complete protection to grouped mice treated with lethal dose of amphetamine (Table 1). This protective effect of reserpine was reduced when the animals were kept at a higher environmental temperature after amphetamine administration and was completely abolished when pretreatment included both reserpine and dopa and the animals were not sedated. Pretreatment of animals with α-methyl-l-tyrosine did not produce any obvious sedation, but was as effective as reserpine in preventing deaths due to the lethal dose of amphetamine. In these animals also, exposure to elevated environmental temperature reduced the protective effect. Preliminary work also showed that when grouped mice were treated either with reserpine or α-methyl-l-tyrosine (both without amphetamine) and kept at the elevated environmental temperature no mortality occurred over 8 hr.

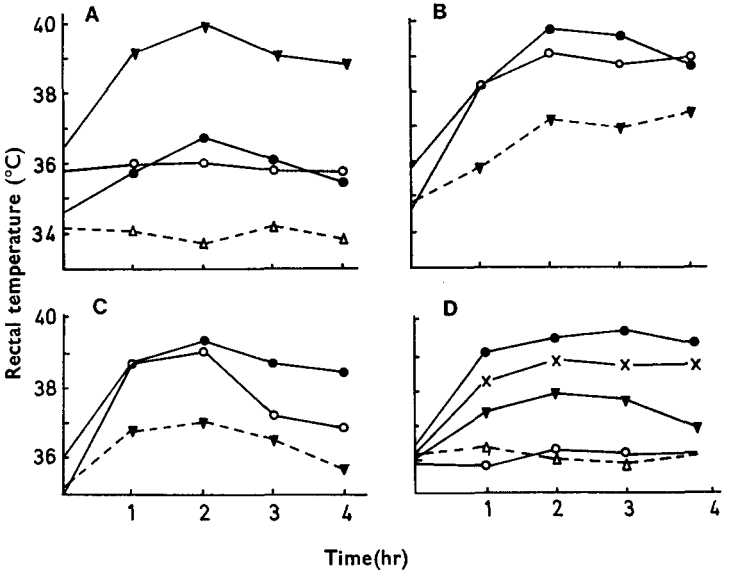

FIG. 1. The influence of reserpine on the effect of amphetamine on the rectal temperature of albino mice kept at an elevated temperature of 38·5°, or pretreated with dopa or α-methyl-l-tyrosine. A. ○—○ Solvent, ▼—▼ solvent + amphetamine, ●—● reserpine + amphetamine, △—△ reserpine. B. ○—○ Solvent + elevated temperature, ▼—▼ reserpine + elevated temperature, ●—● reserpine + amphetamine + elevated temperature. C. ○—○ Solvent + dopa, ▼—▼ reserpine + dopa, ●—● reserpine + dopa + amphetamine. D. ○—○ Solvent of α-methyl-l-tyrosine (AMT), △—△ AMT, ▼—▼ AMT + amphetamine, ×—× AMT + elevated temperature, ●—● AMT + amphetamine + elevated temperature.

Influence on the amphetamine-induced hyperthermia in aggregated mice. (Fig. 1). Amphetamine (7 mg/kg) produced profound hyperthermia in aggregated mice and the animals died when the body temperature exceeded 40°. Reserpine alone produced marked hypothermia in mice and this effect was antagonized by amphetamine, but no hyperthermia was observed in this group of mice. When the reserpinized animals were subjected to elevated environmental temperature, the rectal temperature began rising within the first hour and a rise of 2° in temperature above normal values was observed in 2 hr. No further increase was noted. When the reserpinized animals were treated with amphetamine and then subjected to elevated temperature, the rise in body temperature was comparable to those groups of animals treated with amphetamine only. Administration of dopa caused the body temperature of reserpine-treated mice to return to normal; administration of the 7 mg/kg dose of amphetamine to these grouped mice caused a hyperthermia which was less intense than the control group of animals treated with amphetamine only.

Treatment with α-methyl-1-tyrosine did not produce any significant change in the rectal temperature of aggregated mice. Administration of amphetamine to α-methyl-1-tyrosine-treated animals caused only mild hyperthermia. When these animals were kept at a higher environmental temperature, hyperthermia was observed, the effect being comparable to those obtained in unprotected aggregated mice treated with amphetamine.

Noradrenaline and 5-HT *content of whole brain of aggregated mice* (Table 1). Reserpine (1 mg/kg), 20 hr after administration, caused a 60% reduction of the noradrenaline content of whole brain of aggregated mice (group III). This effect remained unchanged when the reserpinized animals were treated with amphetamine (group IV) or subjected to elevated temperature (group V) or a combination of these two (group VI). Treatment of reserpinized mice with dopa (group VII) partially replenished the noradrenaline content. In these aggregated mice the noradrenaline content of brain was only 25% below control values (group I). Amphetamine (group VIII) did not cause any further change in the above group. α-Methyl-1-tyrosine caused a lowering of brain noradrenaline by 60% (group X) and, as with reserpine, when the α-methyl-1-tyrosine treated animals were given amphetamine (group XI) subjected to elevated temperature (group XII), or were treated with a combination of these two procedures (group XIII), no further change in the noradrenaline level was observed.

Reserpine, but not α-methyl-1-tyrosine, lowered brain 5-HT and other procedures did not alter this amine content further.

Discussion

In aggregated mice, the 7 mg/kg dose of amphetamine killed all the animals. These animals showed marked hyperthermia and the time of death in most instances coincided with the rise of temperature beyond 40°. A significant decrease in brain noradrenaline occurred. Elevation of body temperature has been reported by Greenblatt & Osterberg (1961),

and Askew (1962), and decreased brain noradrenaline content by Maxwell (1959) and Moore (1963) in amphetamine-treated aggregated mice. Although the possibility that these two factors are interrelated cannot be excluded, there is some purpose in seeking which of the two is more directly responsible for the mortality of the animals.

Reserpine offered complete protection to aggregated mice against the toxic effect of amphetamine. In reserpine-treated animals amphetamine did not produce hyperthermia. Amphetamine did not affect the brain noradrenaline concentration already lowered by reserpine. It seems that pretreatment with reserpine prevented the amphetamine-induced release of noradrenaline from the storage sites. These findings suggest that the protective effect of reserpine is dependent on one or both of these factors.

After amphetamine treatment and aggregation, when the reserpinized animals were kept at an elevated environmental temperature, the noradrenaline content of brain did not change, but the animals showed hyperthermia, and this change markedly reduced the protective effect of reserpine. Administration of dopa effectively counteracted the hypothermic effect of reserpine and also partly replenished the brain noradrenaline. Although in these animals there was no hyperthermia and the brain noradrenaline was only 75% of normal values, the amphetamine toxicity was markedly increased.

It can therefore be concluded that if the hyperthermic effect of amphetamine is blocked, a return to normal of the noradrenaline content in reserpine-treated mice abolished the protective effect offered by this tranquillizer against amphetamine toxicity to aggregated mice.

Further support for the important role played by the lowered brain noradrenaline in the reserpine effect is provided by the experiments with α-methyl-1-tyrosine. Both reserpine and α-methyl-1-tyrosine lowered the noradrenaline level to the same extent whereas reserpine also caused a lowering of brain 5-HT. The degree of protection offered to mice by both these drugs was the same. These results indicate that in its protective action against amphetamine toxicity in aggregated mice, reserpine's lowering of brain noradrenaline alone is sufficient. These findings, as well as our recent experimental data showing that the pharmacological effects of reserpine are enhanced in α-methyl-1-tyrosine-treated animals (Menon, Dandiya & Bapna, 1967), support the contention of Carlsson & others (1957) and Carlsson (1961) that reserpine sedation may probably be more directly related to the loss of adrenergic transmitter from the hypothalamus reticular formation and other areas in the brain.

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