Stimulus-dependent antagonism of the α-methyltyrosine-induced lowering of brain catecholamines by (+)-amphetamine in intact mice

SIR,—Littleton (1967) reported that amphetamine retards the decline of brain catecholamines that follows inhibition of their biosynthesis. We wish to confirm his observation and extend it to demonstrate that this antagonism of α-methyltyrosine by (+)-amphetamine is stimulus-dependent.

Male white Swiss mice born within the same two-day period were weaned at four weeks of age and were individually housed for 8 weeks in a quiet air-conditioned room (27°) containing neither females nor any other species of animal. Such mice become hyperexcitable and after being caged with another male mouse will normally engage in intense fighting within about a minute. Care was taken to cause minimal undesigned disturbance to the animals. Mice were given 80 mg/kg DL-α-methyltyrosine intraperitoneally in 0·2 ml of a 0·9% saline solution at pH 2·5. Exactly 1 hr later they were given either 0·1 ml of a 5 mg/kg solution of (+)-amphetamine sulphate in 0·9% saline at pH 7·0, or vehicle alone. At this time, half of those receiving each treatment were quietly returned to their original cages. All others were placed in a strange cage with another of their kind to fight. The mice were decapitated exactly 30 min after injection. Ten non-fighting and ten fighting mice received (+)-amphetamine only; ten non-fighting and ten fighting mice receiving no drug were killed as baseline controls. Whole brains, exclusive of the *bulbus olfactorius* were removed, weighed, and frozen on dry ice within 2 min of death; they were stored at −20°. Individual brains were analysed for noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) (Welch & Welch, 1967).

Table 1 shows that (+)-amphetamine significantly slowed the decline of whole brain noradrenaline and dopamine which was otherwise induced by (±)-α-methyltyrosine in the fighting mice, but that it did not have this effect in the mice which were not put together to fight. Semi-quantitative behavioural observations supported both the suggestion that α-methyltyrosine prevents supra-normal excitation by (+)-amphetamine (Weissman & Koe, 1965; Weissman, Koe & Tenen, 1966; Mennear & Rudzik, 1966; Dingell, Owens & others, 1967; Hanson, 1967), and the suggestion that (+)-amphetamine may temporarily counteract or delay the onset of the behavioural impairment that otherwise follows administration of α-methyltyrosine (Moore & Rech, 1967a; Poschel & Ninteman, 1966). In non-drug mice, brain amines may be either elevated or lowered by fighting, depending upon its intensity (Welch, 1967; Welch & Welch, 1967). Fighting among non-drug controls slightly lowered all three amines in this experiment (footnote Table 1); nevertheless, brain catecholamines were about the same in fighting and non-fighting mice receiving α-methyltyrosine alone. Fighting mice receiving (+)-amphetamine but no pretreatment were becoming uncoordinated or marginally ataxic (or both) by 30 min and their brain catecholamines were much lowered; on the other hand, those that did not fight were "normal" or were only very mildly activated at the time of death, and their brain dopamine and 5-HT were significantly elevated. 5-HT was significantly higher in all mice receiving amphetamine than in those receiving only α-methyltyrosine.

Weissman & others (1966) explained the ability of α-methyltyrosine to prevent, or rapidly stop, the supra-normal excitation normally seen after (+)-amphetamine by summarizing evidence: that the excitatory action of (+)-amphetamine is dependent upon the release of catecholamines to the outside of the neuron; that the availability of catecholamines for release is dependent upon maintenance of the functional pool; that α-methyltyrosine compromises the

TABLE 1. EFFECTS OF (+)-AMPHETAMINE UPON BRAIN CATECHOLAMINES IN FIGHTING AND NON-FIGHTING MICE PRETREATED WITH (±)-α-METHYLTYROSINE*

	1 hr α-MT, 30 min (+)-Amphet. as % of 1 hr α-MT, 30 min saline (1 hr α-MT, 30 min (+)-Amphet. as % of 1 hr saline, 30 min saline)		
	Non-Fight	Fight	P<
Noradrenaline ..	97 ± 2	110 ± 3†	0·001
	(72 ± 2)‡	(87 ± 4)‡	(0·01)
Dopamine ..	97 ± 3	115 ± 5†	0·005
	(77 ± 1)‡	(86 ± 1)‡	(0·001)
5-HT	122 ± 4†	129 ± 7†	n.s.
	(114 ± 2)‡	(117 ± 6)‡	(n.s.)

* α-MT was administered at 80 mg/kg, i.p. and (+)-amphetamine at 5 mg/kg, i.p. Each value in the Table represents a mean ± s.e.m. of 9–11 percentages based upon 9–11 pairs of male mice. Data were evaluated by analysis of variance or by the Wilcoxon Two-Sample Test, depending upon their distribution. Ten non-fighting and ten fighting saline controls, respectively, averaged (in ng/g ± s.e.m.): noradrenaline = 374 ± 14, 341 ± 8 ($P < 0·05$); dopamine = 858 ± 40; 811 ± 33 (n.s.); 5-HT = 869 ± 58; 852 ± 49 (n.s.). Ten non-fighting and ten fighting mice which received (+)-amphetamine but no pretreatment, respectively, averaged (as a percentage of their corresponding controls ± s.e.m.): noradrenaline = 95 ± 3; 81 ± 3 ($P < 0·001$); dopamine = 111 ± 4; 88 ± 2 ($P < 0·001$); 5-HT = 131 ± 3, 117 ± 4 ($P < 0·05$); noradrenaline and dopamine were significantly lower than controls in the fighting mice, viz. $P < 0·001$ and $P < 0·01$, respectively; 5-HT was significantly elevated in both the non-fighting and fighting mice, viz. $P < 0·01$ and $P < 0·05$, respectively.

† α-MT, (+)-amphetamine values were significantly different (at least $P < 0·05$) from the α-MT, (+)-amphetamine values of which they are here expressed as a percentage.

‡ α-MT, (+)-amphetamine values were significantly different (at least $P < 0·05$) from the saline, saline controls of which they are here expressed as a percentage.

functional pool by inhibiting biosynthesis. One might reason that *the activating effect of (+)-amphetamine is largely dependent upon nervous stimulus to effect the release of catecholamines.* If this is so, it will help to explain the synergism between nervous activity and (+)-amphetamine that occurs in normal animals (Moore, 1964; Welch & Welch, 1966); and it will explain the seemingly paradoxical observations that although amphetamine easily penetrates into the brain (Axelrod, 1954), it has no effect upon behaviour or upon the electroencephalograph in animals with lesions in the mid-brain reticular formation (*cerveau isolé*) (Bradley & Elkes, 1957).

In both of the published studies in which (+)-amphetamine antagonized the α-methyltyrosine-induced impairment of behavioural performance, the animals were in stimulus situations, e.g. in one, rats were performing at 20% of normal in a conditioned avoidance response situation (Moore & Rech, 1967a); and in the other, rats had just concluded an 8 hr session of hypothalamic self-stimulation (Poschel & Ninteman, 1966). It is probable that the (+)-amphetamine antagonism of α-methyltyrosine-induced behavioural depression reported by these authors was the result of a retarded decline in brain catecholamines such as that observed by Littleton (1967) and by ourselves.

Further, pretreatment with a monamine oxidase inhibitor similarly retards the behavioural depression and the lowering of brain catecholamines caused by α-methyltyrosine (Moore & Rech, 1967b), and enhances the facilitating effect of amphetamine upon hypothalamic self-stimulation (Stein, 1964). We suggest the working hypothesis that *stimulus itself may normally act in some way to continually modulate the degree of inhibition of monoamine oxidase*, thereby exerting a fine control over the amount of neurotransmitter available in the functional pool for release by nerve stimulation. On this basis, our results may be explained by assuming that, in this experiment, (+)-amphetamine slightly enhanced nervous activity by preventing re-uptake (Glowinski & Axelrod, 1965) and, thereby, indirectly increased the degree of natural inhibition of monoamine oxidase; presumably the observed retardation of tissue catecholamine lowering

by α-methyltyrosine was the result of a net savings of neurotransmitter from oxidative deamination in excess of the increased amount released by the enhanced nervous activity. In the mice receiving only (+)-amphetamine, the increased transmitter release from nerve terminals caused by fighting and the increased extraneuronal longevity of the transmitter caused by (+)-amphetamine, acted in positive feedback manner, one upon the other, to produce the intense behavioural activation and the marked lowering of brain amines commonly associated with the effect of (+)-amphetamine. Undoubtedly the rate of release exceeded total biosynthesis, for if this were not the case catecholamine levels would not have been reduced. Even in the mice that were not stimulated by fighting, there was a small tonic release of neurotransmitter, and the (+)-amphetamine prolonged its action, resulting in a mild increase in nervous activation, a slight natural inhibition of monamine oxidase, and an elevation of dopamine and 5-HT. (With smaller doses of amphetamine or with mice that have not been isolated long enough to be quite so responsive to handling, all three amines may be increased; presumably in this experiment the rate of noradrenaline release had just begun to exceed the supply.)

The concept of continuous massive biosynthesis and oxidative deamination of brain biogenic amines as a means of ensuring their availability in amounts in excess of normal needs has previously been suggested by Brodie & Beaven (1963); however, they regarded the rate of synthesis and the rate of breakdown as constant, save that the latter was temporarily diminished when nervous stimulus released transmitter amines from the nerve ending, thus making them unavailable for catabolism within the neuron. Our suggestion differs in that we propose that the level of monoamine oxidase activity may be modulated by stimulus.

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