Development of tolerance to the behavioural depressant effects of α-methyltyrosine

SIR,—α-Methyltyrosine produces marked behavioural depression in animals. It disrupts conditioned avoidance behaviour in rats, guinea-pigs and cats (Hanson, 1965; Moore, 1966). It also disrupts rotarod performance and spontaneous locomotor activity (Rech, Borys & Moore, 1966), operant behaviour (Schoenfeld & Seiden, 1967) and self-stimulation (Poschel & Ninteman, 1966) in rats. The behavioural depressant effects of α-methyltyrosine appear to be associated with the ability of this compound to deplete the brain of catecholamines (Moore & Rech, 1967; Rech, Carr & Moore, 1968).

Despite the pronounced behavioural depression observed after acute dosage of α-methyltyrosine in animals, minimal central depressant effects were observed after the administration of the drug to man (Charalampous & Brown, 1967; Gershon, Hekimian & others, 1967; Engelman, Horwitz & others, 1968). This lack of central depression after large doses of the drug is puzzling in light of the animal findings. I wondered if the acute findings in animals were maintained after chronic dosage.

Male albino mice (Spartan Farms), 25–30 g were maintained (8 per cage) on a ground diet (Wayne Lab-blox) containing 0, 0·3 or 1% L-α-methyltyrosine. At various times after the feeding program was initiated, spontaneous locomotor activity was measured in circular actophotometer cages (Woodward Research Corporation). Two mice were placed in each cage. After a 10 min period of acclimatization, motor activity was recorded for 10 min. The mice were then decapitated and blood collected from the trunk into beakers containing heparin. Four brains were pooled and analysed for noradrenaline and dopamine (Moore & Rech, 1967). Four plasma samples were pooled and analysed for α-methyltyrosine (Carr & Moore, 1968).

Table 1 summarizes the effects of a 24 hr diet of 0·3 and 1·0% α-methyltyrosine. The results depicted here essentially confirm those reported by Johnson, Kim & others (1967). That is, when administered in the diet, the drug produced a dose-related reduction of spontaneous locomotor activity. This behavioural effect was mirrored by a reduction in the brain contents of noradrenaline and dopamine. The 1% diet, but not the 0·3% diet, of α-methyltyrosine markedly reduced both food intake and body weight. Accordingly, the 0·3% diet was used in all subsequent experiments.

Fig. 1 shows the effects of a chronic diet of α-methyltyrosine on spontaneous locomotor activity, plasma α-methyltyrosine and brain catecholamine levels. The values depicted for day 1 are similar to those presented for the 0·3% diet

TABLE 1. EFFECTS OF A 24 HR DIET OF α-METHYLTYROSINE IN MICE

			α-Methyltyrosine	
	N	Control	0·3%	1·0%
Motor activity (counts/10 min) ..	16	504 ± 18	212 ± 16*	53 ± 9*
Brain noradrenaline (μg/g)	8	0·35 ± 0·01	0·20 ± 0·01*	0·13 ± 0·01*
Brain dopamine (μg/g)	8	0·71 ± 0·05	0·42 ± 0·04*	0·22 ± 0·04*
% change in body wt	4	+1·5	−0·3	−12·1
Food intake (g/g body wt)	4	0·178 ± 0·020	0·177 ± 0·018	0·079 ● 0·006*
α-Methyltyrosine intake (mg/kg) ..	4	—	530	785
Plasma α-methyltyrosine (μg/ml) ..	8	—	6·6 ± 0·4	52·8 ± 8·9

Figures represent the mean ±1 standard error as determined from 4 cages of 8 animals on each diet. N, the number of determinations.
* Significantly different from control diet at $P < 0·01$.

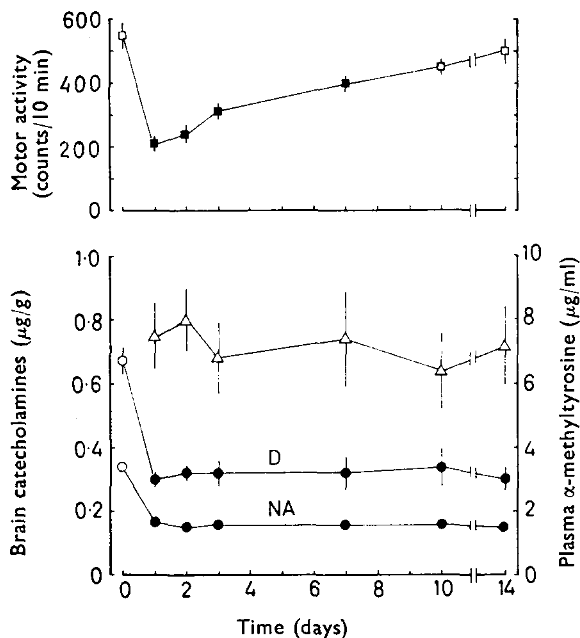

FIG. 1. Effects of a chronic diet of 0·3% α-methyltyrosine on spontaneous locomotor activity, plasma levels of α-methyltyrosine and brain levels of catecholamines. Plasma α-methyltyrosine (△) and brain cateholamines (○) represent the mean of 8 determinations and motor activity (□) represents the mean of 16 determinations; the *vertical lines projected upon each point represents one standard error of that mean.* Solid points represent those values that are significantly different from control (zero time) at $P < 0{\cdot}01$.

in Table 1. Continued administration of the drug for up to 7 days resulted in significantly lower levels of motor activity. However, this depressant effect diminished with time so that by 10 days and thereafter there was no significant reduction of motor activity. Throughout the two week period there was no alteration in the plasma content of α-methyltyrosine and brain catecholamine levels remained low.

It would seem important to determine the mechanism of tolerance development to the behavioural effect of α-methyltyrosine. Its metabolites (α-methyldopamine or α-methylnoradrenaline) may slowly accumulate in the brain and as "false transmitters" gradually assume the functions of the depleted noradrenaline and dopamine. An alternative explanation would be that during the development of tolerance "central adrenergic receptors" develop an increased sensitivity to noradrenaline. However, regardless of the mechanism, the development of tolerance to the depressant effects of the drug may account for the lack of marked sedative and antipsychotic effects observed when it is administered chronically to man.

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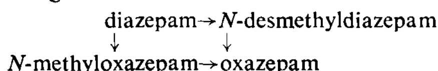
Metabolism of diazepam in isolated perfused liver of rat and mouse

SIR,—It was reported (Schwartz, Koechlin, & others, 1965; Schwartz, Bommer & and Vane, 1967) that in rats, dogs and man the major metabolic pathways of ^{3}H-labelled diazepam involved *N*-demethylation and C-3 hydroxylation, according to the following scheme:



We now confirm the formation of these metabolites by using the isolated perfused liver of rats and mice.

Male Sprague-Dawley rats (200 g) or male Swiss mice (21–24 g) were used as donors of blood and livers. The animals were kept on a standard diet (ALAL 56) and had food and water *ad libitum*. Diazepam or its metabolites were added to the perfusion fluid in concentrations of 50 μg/ml. Details of the technique for the perfusion of the isolated livers of rats and mice have been described elsewhere (Kvetina & Guaitani, 1968). At selected intervals, 2 ml amounts of the perfusion fluid were withdrawn from the circulation and extracted twice with 10 ml of ether. The combined ether extracts, of diazepam and its metabolites were evaporated to dryness.

The residue was dissolved in hexane and quantitatively transferred into a thin-layer plate of Silica Gel G. Pure standards of diazepam and its three metabolites were run alongside the sample extracts for identification of the compounds.

The plates were developed to 15 cm above the origin in glass tanks using the solvent system chloroform acetone, 90:10. After development, the plates were allowed to dry completely and then viewed under ultraviolet light (245–350 mμ) to identify the compounds on the plate.

The metabolites formed during the liver perfusion were further identified and verified by two-dimensional chromatography in at least three solvent system pairs (chloroform–heptane–ethanol (10:10:1), heptane–chloroform–acetic acid–ethanol (5:5:1:0·3), chloroform–acetone (90:10)).

The metabolite identification was reinforced by the colour reactions developed