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Effect of chronic intoxication with (+)-amphetamine on its concentration in liver and brain and on [^{14}C] leucine incorporation into microsomal and cytoplasmic proteins of rat liver

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Rats were treated with increasing concentrations of (+)-amphetamine sulphate in drinking water for 90 days. The ingested dose of amphetamine was found to increase from 16 mg kg^{-1} on the first day up to 90 mg kg^{-1} on the 32nd day of treatment. The rats were maintained on the highest dose regime for a further 58 days without any deaths, which showed that tolerance to the overall toxicity of the drug developed. The concentrations of [^{3}H]amphetamine in liver and brain of chronically treated rats were significantly higher than those of controls. Chronic treatment with amphetamine significantly reduced body and liver weight of rats, but did not influence the relative liver to body weight. A marked inhibition of [^{14}C]leucine incorporation into liver microsomal and cytoplasmic proteins was observed after 90 days of treatment with amphetamine. The relation between inhibition of microsomal protein synthesis and the increase of amphetamine concentrations in liver and brain is discussed.

We have previously studied the effect of chronic (+)-amphetamine sulphate treatment on food intake, body weight, locomotor activity and subcellular distribution of the drug in rat brain (Magour, Coper & Fähndrich, 1973). It was evident that in long term treatment with increasing doses of (+)-amphetamine tolerance develops to the toxic and anorexigenic effects of the drug. This observation suggested a possible alteration in the rate of elimination of amphetamine in chronically treated rats. Liver enzymes are established as the major site of metabolism of amphetamine in the rat (Axelrod, 1954), we therefore tested the effect of chronic treatment with amphetamine on its concentration in liver and brain and on protein synthesis in rat liver microsomes.

MATERIALS AND METHODS

(+)-[^{3}H]Amphetamine sulphate (6·2 Ci m mol^{-1}; NEN Chemicals), L-[U-^{14}C]leucine (342 mCi m mol^{-1}, Amersham); unlabelled (+)-amphetamine sulphate (Merck AG). Female Wistar rats, 120–150 g, in 4 groups of 6 rats each were kept on a day-night schedule of 5 a.m.–5 p.m. The two control groups had free access to food and water and the two amphetamine-treated groups had the water replaced by amphetamine solution dissolved in tap water. The concentrations of amphetamine were 0·01, 0·02, 0·03, 0·04 and 0·05% in the 1st, 2nd, 3rd, 4th, and 5th week of treatment. The rats were then kept on 0·05% amphetamine for another 55 days. Body weight, food and amphetamine consumptions were measured daily between 9·00–10.00 a.m.

Amphetamine concentration in liver and brain

Six control and 6 amphetamine-treated rats were injected intraperitoneally with 200 μCi kg^{-1} [^{3}H]amphetamine plus 4 mg kg^{-1} unlabelled (+)-amphetamine sulphate and were decapitated after 1 h. Livers and brains were immediately removed, weighed and rinsed in 0·9% saline. Unchanged amphetamine was extracted using the method of Axelrod (1954) as modified by Maickel, Cox & others (1969). The recovery was 92–95%. Aliquots (0·5 ml) of the acid phase containing [^{3}H]amphetamine were mixed with NC*-solubilizer and 15 ml of a scintillation cocktail (0·4% PPO and 0·005% POPOP in toluene). Samples were counted in a Packard Liquid Scintillation Spectrometer (3380) using an external standard for correction of quenching.

Incorporation studies

Six control and 6 amphetamine-treated rats were fasted for 18 h and then they were injected with 25 μCi kg^{-1} [U-^{14}C]leucine (i.p.) and decapitated after 60 min. The livers were removed, weighed and rinsed in 0·9% saline. Microsomal and cytoplasmic fractions were isolated and washed as described by Remmer, Greim & others (1967).

Protein extraction and counting

Two ml microsomal suspension or 5 ml cytoplasmic fraction were mixed with an equal volume of 20% trichloroacetic acid (TCA). The samples were kept at room temperature (20°) for 1 h and centrifuged for 10 min. The protein pellets were washed twice with 5% TCA, containing 0·1% unlabelled L-leucine and once at 90° for 15 min. The TCA phases from the cytoplasmic fractions were saved. The remaining pellets were again washed twice with a mixture of chloroform–methanol (1:2) and once at 50° for 10 min and centrifuged. They were finally washed once with ether, centrifuged and kept at room temperature until dry. The dry pellets were dissolved in 2 ml 1 N NaOH and kept in a water bath at 70° for 90 min until clear. Aliquots (0·2 ml) were mixed with 15 ml scintillation cocktail (Bray, 1960) and counted.

RESULTS AND DISCUSSION

Under our experimental conditions the rats ingested increasing doses of amphetamine starting with 16 mg kg^{-1} on the first day up to 90 mg kg^{-1} on the 32nd day of treatment (Fig. 1). They continued to consume the highest dose level (90 mg kg^{-1} day^{-1}) for a

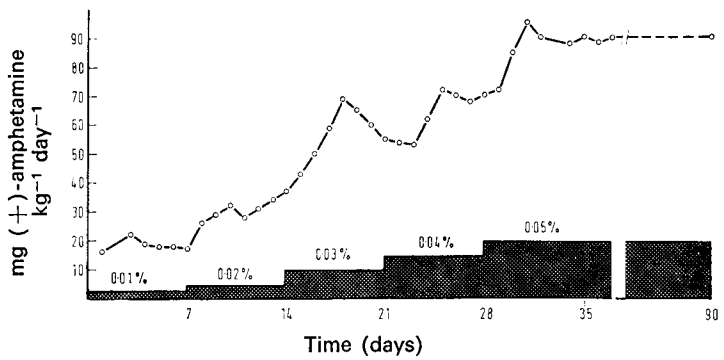

FIG. 1. Average dose of (+)amphetamine ingested daily in drinking water. Shaded area illustrates the concentration of amphetamine used each week. Each point is the average of 12 rats.

* Nuclear Chicago.

further 58 days without any deaths suggesting that tolerance may have developed to the overall toxicity of the drug. This could be due to alterations in the elimination rate of (+)-amphetamine in the chronically treated rats (see Magour & others, 1973).

It is generally agreed that liver enzymes are mainly responsible for the metabolism of amphetamine in the rat. Furthermore it has been reported (Dingell & Bass, 1969) that amphetamine is metabolized in the rat isolated perfused liver, but not in microsomal preparations *in vitro*. On the basis of these findings we decided to maintain the *in vivo* conditions by determining the concentration of unchanged (+)-[^{3}H]-amphetamine in liver and brain tissues. As shown in Table 1, the concentration of

Table 1. *Liver and brain levels of* (+)-[^{3}H]*amphetamine in rats pretreated for 90 days with either* (+)*-amphetamine or saline* (*controls*). On the 91st day the rats were injected with 200 μCi kg^{-1} (+)-[^{3}H]amphetamine and 4 mg kg^{-1} unlabelled amphetamine (i.p.), 1 h before decapitation.

	d min^{-1} × 10^{-2} g^{-1} tissue ± s.d. (n = 6)			
	Control	Amphetamine treated	Change (%)	$P<$
Liver ..	2149 ± 432	3797 ± 650	+76	0·001
Brain ..	1636 ± 215	2106 ± 381	+29	0·01

(+)-[^{3}H]amphetamine is significantly increased in liver and brain of chronically treated rats. These results are in agreement with those of Ellison, Okun & others (1971) who observed an elevation of the concentration of unchanged amphetamine in livers of amphetamine tolerant cats 1 h after a single intraperitoneal injection of 10 mg kg^{-1} of (+)-[^{3}H]amphetamine.

Chronic intoxication with (+)-amphetamine significantly reduced both body weight and total liver weight by 37 and 33%, respectively (Table 2). The relative liver

Table 2. *Changes in body and liver weight after rats had been treated for 90 days with* (+)*-amphetamine or saline* (*controls*)*; results* ± *s.d., n = 12.*

	Body weight (g)	Liver weight (g)	*Relative liver weight (g)
Control	253 ± 19	7·47 ± 1·09	2·95 ± 0·27
Amphetamine treated	160 ± 12	4·97 ± 0·88	3·10 ± 0·42
Changes (%)	−37	−33	—
$P<$	0·001	0·005	n.s.

* "Relative liver weight" is the weight of liver calculated per 100 g body weight.

weight, however (as calculated per 100 g rat), is not changed. This observation is consistent with that of Platt & Cockrill (1969) who found no changes in relative liver weight of rats after 14 days of oral application of 25 mg kg^{-1} of (±)-amphetamine.

To find out whether the observed increase of amphetamine concentration in liver and brain and the decrease in body and liver weight were paralleled by a reduction of protein synthesis in the liver, we determined the incorporation of [^{14}C]leucine into liver microsomal and cytoplasmic proteins. Chronic treatment with amphetamine significantly inhibited the incorporation of [^{14}C]leucine per mg of microsomal and cytoplasmic proteins of the liver by 21 and 25%, respectively (Table 3). This effect was more marked when calculated per g liver. The amount of microsomal protein per g liver was also reduced but there was no significant change in the amount of

Table 3. [^{14}C]*Leucine incorporation into microsomal and cytoplasmic proteins of rat liver after the rats had been treated for 90 days with (+)-amphetamine or saline (controls); results ± s.d., n = 6.*

	Microsomal protein			Cytoplasmic protein			Acid soluble activity counts $min^{-1} g^{-1}$ liver
	Counts $min^{-1} mg^{-1}$	mg g^{-1} liver	Counts $min^{-1} g^{-1}$ liver	Counts $min^{-1} mg^{-1}$	mg g^{-1} liver	Counts $min^{-1} g^{-1}$ liver	
Control	1525 ± 334	16,70 ± 1,56	25468 ± 5428	432 ± 65	90,96 ± 5,89	39295 ± 5283	11377 ± 1025
Chronic	1209 ± 204	13,40 ± 1,81	16200 ± 3213	326 ± 93	86,43 ± 2,70	28176 ± 8111	17339 ± 2697
Change (%)	−21	−20	−36	−25	−5	−28	+52
$P<$	0·01	0·02	0·005	0·01	0·1 (n.s.)	0·025	0·001

cytoplasmic protein (Table 3). The observed reduction in amino-acid incorporation into liver proteins did not seem to be dependent on an altered uptake of precursors into the liver, since chronic treatment with amphetamine significantly increased the level of TCA soluble activity in this tissue (Table 3). This effect cannot be attributed to dietary protein deprivation either (von der Decken, 1967) since, under these experimental conditions, the rats have developed a complete tolerance to the anorexigenic effect of the drug after 14 days of treatment (Magour & others, 1973).

Our results suggest that chronic intoxication with amphetamine inhibits protein synthesis by liver microsomes and consequently impairs hepatic metabolizing enzymes which results in an increased concentration of the drug in both liver and brain. This view is supported by the finding that imipramine and desipramine inhibit the metabolism of amphetamine in rat liver which results in higher levels of circulating amphetamine and therefore in higher brain levels of amphetamine (Valzelli, Consolo & Morpugo, 1967; Lemberger, Sernatinger & Kunzman, 1970). As repeated administration of amphetamine results in the development of tolerance to some of its central effects in rats (Kosman & Unna, 1968; Lewander, 1971; Magour & others, 1973), it is evident that tolerance to amphetamine is not associated with stimulated microsomal protein synthesis or with a faster elimination of the drug from liver and brain. This is a further support for the hypothesis that tolerance to amphetamine is likely to be cellular rather than metabolic.

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