stantially different (922 vs 1056 nmoles/100 mg of protein). In addition, there were no significant differences in ALA dehydratase activity between normal and deficient animals when the product, phorphobilinogen, was isolated on Dowex 1-acetate.

Since iron must be maintained in its ferrous state for incorporation by ferrochelatase into protoporphyrin IX [22], the effect of ascorbic acid deficiency on this enzyme was of interest. As can be seen in Table 3, although the quantity of cytochrome P-450 in ascorbic acid deficient animals was markedly decreased (8.3 vs 21.8 nmoles/100 mg of protein), there were no significant differences in ferrochelatase activity (589 vs 536 nmoles/hr/100 mg of protein).

Contrary to the proposal that the initial and rate-limiting steps in heme synthesis might be impaired in ascorbic acid deficiency [9, 10], our results indicate no significant differences in ALA synthetase activity in either whole cell homogenates or sonicated mitochondria. In addition, there were no substantial differences in ALA dehydratase or in ferrochelatase activities. Thus, ascorbic acid deficiency does not affect the activities of the key enzymes involved in heme synthesis. However, the possibility that it might be involved in the synthesis of the apoprotein of cytochrome P-450 or in the degradation of the heme protein should be considered.

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Acute and chronic effects of (−)-amphetamine on seizure threshold and brain catecholamines in mice

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Amphetamine has been employed for over 35 years for the treatment of epilepsy [1, 2]. While the acute and chronic effects of (+)-amphetamine on the seizure susceptibility of rodents have been the subject of numerous publications [3–9], few studies have examined the effects of its (−)-isomer on changes in seizure threshold [4, 8, 9]. We have recently reported that acute administration of (−)-amphetamine at doses of 1.25 to 10 mg/kg increases seizure threshold in mice by 12–49 per cent, while doses of 15–45 mg/kg were without effect [9].

The present study was designed to determine whether (−)-amphetamine-induced changes in pentylenetetrazol (PTZ) seizure threshold (a model system for central excitation) could be correlated with drug-induced alterations in endogenous concentrations of brain norepinephrine and dopamine and their rates of biosynthesis. Two doses of (−)-amphetamine were compared, 4 and 15 mg/kg, the former highly effective in elevating seizure threshold after acute administration and the latter previously observed to be ineffective in this test system [9]. It was also of interest to ascertain whether chronic (−)-amphetamine administration for 7 days could produce alterations in these neuropharmacological and neurochemical parameters, when compared with acute drug treatment.

Materials and methods

Animals. Male albino CD-1 mice (Charles Rivers) weighing 20–30 g were used in this study. The animals were housed in groups of 5–10 mice in cages of 16.5 × 28 cm and permitted free access to food (Purina rat chow) and water. The animal quarters were illuminated for 12 hr, alternating with 12 hr of darkness.

Drug-dosing schedule. Saline and (−)-amphetamine sulfate were administered i.p. in a constant volume of

1 ml/100 g body weight; doses of (−)-amphetamine refer to the sulfate salt. (−)-Amphetamine (4 mg/kg) has been shown previously to produce maximal changes in PTZ seizure threshold when administered 30 min prior to testing [9]. In chronic studies, (−)-amphetamine or saline was administered once daily for 7 consecutive days, with alterations in seizure threshold or neurochemical determinations evaluated 30 min after the final injection on day 7. In both acute and chronic studies, seizure threshold and neurochemical determinations were conducted in separate groups of mice.

Determination of PTZ seizure threshold. PTZ was employed to induce clonic seizures in groups of 10–15 mice, each animal tested only once. The method of Orloff *et al.* [10] was modified as previously described [8]. The mouse was briefly restrained while a 0.5% solution of PTZ was infused into a lateral tail vein at a constant rate of 2.55 mg/min; the endpoint was 3 consecutive sec of clonic seizure activity characterized by jerking movements of the ears and jaw with purposeless running movements of the forelimbs.

Neurochemical determinations. Endogenous concentrations of tyrosine (TY), norepinephrine (NE) and dopamine (DA) and an estimation of the rates of biosynthesis of the catecholamines were determined in single mouse brain samples as described earlier [11]. [3,5-^{3}H]tyrosine ([^{3}H]TY; sp. act. 50 Ci/m-mole) was obtained from New England Nuclear Corp. This material was found to be > 95 per cent pure by spotting on thin-layer chromatography plates and developed using a solvent system of *n*-butanol- acetic acid–water (5:1:4). [^{3}H]TY (3 mCi/kg, i.v.) and (−)-amphetamine were injected 45 and 30 min prior to decapitation respectively. The catecholamines and TY (endogenous and [^{3}H]TY) were extracted from the brain using a 0.4 N perchloric acid reagent. TY was separated from the catecholamines by alumina adsorption, and endogenous concentrations determined fluorometrically (Aminco–Bowman spectrophotofluorometer). Samples containing [^{3}H]TY were purified on a Dowex-50 chromatographic column, and the radioactive TY was quantitated by liquid scintillation spectrometry (Beckman LSC models 345 or 355). NE and DA were eluted from the alumina with 0.05 N perchloric acid and endogenous concentrations were determined fluorometrically following oxidation with iodine reagent. The [^{3}H]NE and [^{3}H]DA formed from [^{3}H]TY were separated on a Dowex-50 chromatographic column and quantitated using liquid scintillation spectrometry. Estimation of the rates of biosynthesis of [^{3}H]catecholamines ([^{3}H]CA) from [^{3}H]TY was calculated by using the following equation:

$$\text{nmoles CA/g } t^{-1} = \frac{\text{dis./min CA/g} \cdot 2}{\text{specific activity [}^3\text{H]TY}}$$

where t = the time interval between the administration of [^{3}H]TY and animal sacrifice. The factor 2 in the numerator corrects for the loss of one tritium atom from the [^{3}H]TY when it is converted to [^{3}H]DA or [^{3}H]NE.

Data analysis. Results are expressed as the mean ± S.E.M. Statistical comparisons of (−)-amphetamine-treated vs control mice were performed using the 95 per cent confidence interval of a ratio [12].

Results and discussion

The results of this study are summarized in Table 1. Acute administration of (−)-amphetamine (4 mg/kg) increased PTZ seizure threshold by 49 per cent, an effect which was attenuated to 31 per cent after seven daily injections of the same dose of (−)-amphetamine. Acute and chronic administration of (−)-amphetamine at a dose of 15 mg/kg failed to significantly alter the seizure threshold.

A single injection of the lower dose of (−)-amphetamine increased endogenous concentrations of NE and DA by 7 and 21 per cent, and enhanced their rates of biosynthesis by 55 and 51 per cent, respectively. On day 7, these responses were reduced or absent. The endogenous concentrations and rates of biosynthesis of whole brain NE were not significantly different in control and drug-treated mice; endogenous DA levels were increased by 14 per cent, while its rate of biosynthesis was nonsignificantly elevated by 14 per cent after chronic drug administration.

The results of this study suggest that the (−)-amphetamine (4 mg/kg)-induced increase in PTZ seizure threshold is mediated indirectly by the central noradrenergic and or dopaminergic systems. This hypothesis is supported by studies in this laboratory demonstrating that pretreatment with α- and β-noradrenergic and dopaminergic receptor blockers prevented or reduced the magnitude of this response [9]. Moreover, pretreatment with reserpine, α-methyltyrosine methylester, the specific dopamine β-hydroxylase inhibitor FLA-63 [13] and 6-hydroxydopa, the carboxyl analog of 6-hydroxydopamine capable of selectively destroying noradrenergic nerve terminals [14, 15], all substantially reduced or abolished the ability of (−)-amphetamine to increase PTZ seizure threshold after acute administration (M. Gerald and T. Gupta, manuscript submitted

Table 1. Effects of acute and chronic administration of (−)-amphetamine on pentylenetetrazol seizure threshold, endogenous mouse brain catecholamines, and their rates of synthesis.*

Treatment	Pretreatment (days + 30 min)	Seizure threshold of PTZ (mg/kg)	Norepinephrine Endogenous (μg/g)	Norepinephrine Synthesis (nmoles/g × min^{-1} × 10^{-2})	Dopamine Endogenous (μg/g)	Dopamine Synthesis (nmoles/g × min^{-1} × 10^{-2})
Saline	1	39.9 ± 1.4	0.50 ± 0.01	1.37 ± 0.05	1.10 ± 0.09	2.87 ± 0.11
	7	42.1 ± 0.5	0.50 ± 0.01	1.35 ± 0.07	1.10 ± 0.02	2.88 ± 0.13
(−)-Amphetamine (4 mg/kg)	1	58.6 ± 2.8†	0.53 ± 0.01‡	2.15 ± 0.19‡	1.33 ± 0.06‡	4.34 ± 0.37‡
	7	55.3 ± 3.8†	0.50 ± 0.01	1.50 ± 0.15	1.25 ± 0.02‡	3.27 ± 0.14
(−)-Amphetamine (15 mg/kg)	1	40.8 ± 1.8	0.46 ± 0.01§	1.49 ± 0.11	1.37 ± 0.04‡	4.06 ± 0.28‡
	7	40.0 ± 1.7	0.48 ± 0.01	1.87 ± 0.18‡	1.32 ± 0.03‡	4.84 ± 0.40‡

* Seizure threshold or neurochemical determinations were carried out 30 min after a single injection (day 1) or after seven consecutive daily i.p. injections (day 7) of (−)-amphetamine or saline. Values represent the mean ± S.E.M. N = 10–15 in seizure studies; N = 12 and 20–24 in drug- and saline-treated mice, respectively, in neurochemical studies.

† Significant increase in seizure threshold ($P < 0.05$).

‡ Significantly higher than corresponding controls ($P < 0.05$).

§ Significantly lower than corresponding controls ($P < 0.05$).

for publication). The metabolite *p*-hydroxynorephedrine has been proposed to mediate the development of tolerance to (+)-amphetamine [16, 17]. It has been shown that (−)-amphetamine is not metabolized to this compound [18], nor is *p*-hydroxynorephedrine an amphetamine metabolite in mice [19]. While tolerance was not acquired to the increase in PTZ seizure threshold elicited by (−)-amphetamine, this effect was decreased after chronic administration. These effects might be the consequence of a reduction of noradrenergic and/or dopaminergic influences, as reflected by nonsignificant increases in the rates of biosynthesis of these catecholamines after chronic (−)-amphetamine administration. At this time, we cannot exclude the possibility that this reduction is the consequence of diminished or altered central receptor sensitivity [20, 21].

Acute administration of 15 mg/kg of (−)-amphetamine elicited a slight reduction in NE levels and increased the concentration and rate of synthesis of DA by 24 and 42 per cent, respectively. After chronic administration, NE levels returned to normal, while its rate of synthesis was increased by 38 per cent; endogenous DA concentrations remained increased by 20 per cent and its biosynthetic rate enhanced to 68 per cent above control. While the neurochemical profile observed after chronic administration of 15 mg/kg was very similar to that observed after a single dose of 4 mg/kg, these changes were not accompanied by corresponding elevations in seizure threshold. At this time, it is not clear whether the neurochemical changes in whole brain catecholamines after these high doses of (−)-amphetamine (15 mg/kg) represent an accurate portrayal of the dynamic changes occurring at the central neuronal receptor sites [22].

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