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AMPHETAMINE TOXICITY IN HYPERTHYROID MICE: EFFECTS ON ENDOGENOUS CATECHOLAMINES*

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Abstract—The actions of *d*-amphetamine were studied in mice made hyperthyroid by daily injections of triiodothyronine. The LD_{50} of *d*-amphetamine in hyperthyroid mice was approximately one twentieth of that in euthyroid animals. *d*-Amphetamine induced a dose-dependent reduction of norepinephrine stores in the brain, heart, and spleen of euthyroid mice. The *d*-amphetamine-induced depletion of norepinephrine was significantly greater in the brain and spleen of hyperthyroid mice. *d*-Amphetamine had no effect on the catecholamine content of adrenal gland of hyperthyroid mice. Chlorpromazine, phenoxybenzamine, and propranolol pretreatment reduced the lethality of *d*-amphetamine in hyperthyroid mice while α-methyl-*m*-tyrosine, α-methyl-*p*-tyrosine, and reserpine pretreatment did not. The toxicity of α-methyl-*m*-tyrosine was enhanced in hyperthyroid mice.

The role of endogenous catecholamines in the enhanced toxicity of *d*-amphetamine and α-methyl-*m*-tyrosine in hyperthyroid animals is discussed in the light of these findings.

THE toxicity of amphetamine is influenced by many environmental factors such as temperature, noise, crowding (aggregation), painful stimuli, and forced exercise.[1–6] These factors probably exert their influences by effecting changes in pituitary–hormone systems and/or the autonomic nervous system. Previous studies have implicated norepinephrine released from endogenous stores as playing a role in the enhanced toxicity of *d*-amphetamine in aggregated mice[7, 8] and stressed rats.[6, 9] Adrenal steroids[10] and thyroid hormones[11] also influence the toxicity of amphetamine.

Askew[2] reported that repeated injections of thyroxine increased the toxicity of amphetamine in aggregated mice. Halpern *et al.*[11] showed that the same was true in individually caged mice. Hyperthyroid animals are known to be more sensitive than euthyroid animals to the toxicity of many substances and in particular to drugs that mimic or potentiate the actions of the sympathetic nervous system. In addition to amphetamine, the toxicity of monoamine oxidase inhibitors,[12] imipramine,[13] desmethylimipramine,[14] and ephedrine[11] are all increased in hyperthyroid animals. Since all of these drugs can influence the actions of catecholamines and, since the mutual potentiation of thyroid–catecholamines is well documented (e.g. Ref. 15), it appears reasonable to assume that the enhanced toxicity of *d*-amphetamine in hyperthyroid mice is at least in part a consequence of thyroid–catecholamine interactions.

The toxicity and norepinephrine-depleting actions of *d*-amphetamine are enhanced in aggregated mice; drugs that intefere with the actions or storage of endogenous catecholamines modify the toxicity of *d*-amphetamine.[7, 8] As a result of these findings,

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it was proposed that norepinephrine which is released from endogenous stores plays an important role in the mechanisms leading to the death of aggregated mice treated with *d*-amphetamine. By a similar approach, the present study attempts to show that norepinephrine also plays a role in the enhanced toxicity of *d*-amphetamine in hyperthyroid mice.

METHODS

Male albino mice (Charles River Mouse Farms) weighing 24–30 g were used throughout this study. They were made hyperthyroid by i.p. injections of l-triiodothyronine (5 mg/kg) on three consecutive days. During this time they were housed in large cages in groups of 24. On the fourth day they were injected with *d*-amphetamine; just prior to the injection, total-body oxygen consumption of some mice was determined by a volume meter (model 160, Med-Science Electronics, St. Louis, Mo.). In the drug protection studies the mice were injected with the drugs at various times after the last triiodothyronine injection and before the administration of *d*-amphetamine. After the injection of *d*-amphetamine, the mice were placed individually in small wire mesh cages and kept in a room where the circulating air was maintained at a temperature of 24 $\pm$ 0·5°.

When tissue catecholamines were determined, the mice were sacrificed 1·5–2 hr after the *d*-amphetamine injection. Brain, heart, and spleen norepinephrine levels were determined in extracts prepared from the tissues of four mice; adrenal catecholamines were determined on paired adrenals from individual mice. The method of analysis was carried out as described previously[7] except that, instead of utilizing chromatographic columns, the extracts were shaken with alumina in glass-stoppered centrifuge tubes.

All drug solutions were prepared immediately prior to their use and adjusted so that the prescribed dose was injected i.p. in a volume of 1 ml/100 g body weight. Triiodothyronine was dissolved in a small amount of 0·1 N sodium hydroxide, the pH adjusted to 10, and the solution made up to volume with water. The salts in which the doses of the drugs are expressed and the solvents used to dissolve them are as follows : *d*-amphetamine sulfate, α-methyl-*m*-tyrosine, propranolol hydrochloride, and chlorpromazine hydrochloride were dissolved in water; α-methyl-*p*-tyrosine was dissolved in 5 N NaOH, neutralized to pH 10 with 1 N HCl, and diluted to volume with water; crystalline reserpine was dissolved in acetic acid and diluted to volume with water; phenoxybenzamine hydrochloride was suspended in 0·5% methyl cellulose or dissolved in propylene glycol (final concentration 10%) and diluted to volume with water.

Norepinephrine values were compared by Student's *t* test and the per cent mortality data by the chi-square 2 $\times$ 2 contingency analysis.

RESULTS

Toxicity of d-*amphetamine in hyperthyroid mice*

The daily injections of triiodothyronine produced no grossly apparent effects. Although the oxygen consumption of the mice was increased by 35%, the animals maintained their body weight during the four-day treatment period. In one study the average weight of 50 mice on the four consecutive treatment days was : 27·4, 27·1, 27·2, and 26·8 g.

In both euthyroid and hyperthyroid mice the i.p. injection of 5 or 10 mg *d*-amphetamine/kg caused excitement and increased motor activity. In hyperthyroid but not in euthyroid animals this initial period of hyperactivity was followed by within 1 to 2 hr a period of depression or exhaustion, which became progressively more pronounced and terminated in death. The per cent mortality in the triiodothyronine-pretreated mice was dependent on the dose of *d*-amphetamine.

A typical per cent mortality–time curve is depicted in Fig. 1. The majority of animals died between the first and the second hour after injection. Based upon the

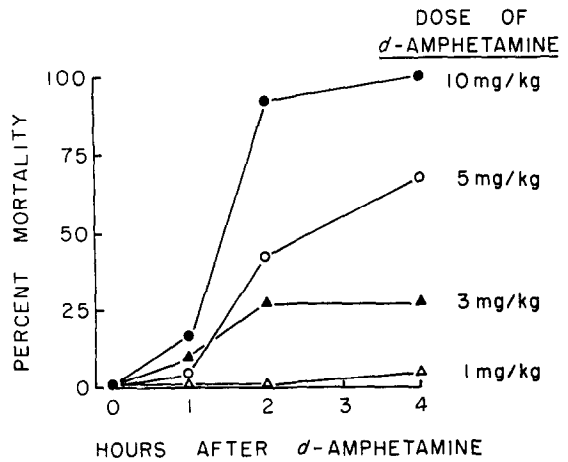

FIG. 1. Time mortality curve for *d*-amphetamine in hyperthyroid mice. The value at each dose was based upon the results from 24 injected mice.

number of mice dead in 4 hr, the LD_{50} and 95% fiducial limits for *d*-amphetamine in triiodothyronine-pretreated mice was 4·0 (3·3–4·8) mg/kg. In euthyroid mice the LD_{50} for *d*-amphetamine was 98 (90–107) mg/kg; there were no deaths with 10 mg *d*-amphetamine/kg.[7] Thus triiodothyronine pretreatment enhances the lethality of *d*-amphetamine some 20- to 25-fold.

Effect of d-*amphetamine on the tissue catecholamine levels of hyperthyroid mice*

It has been proposed that endogenous catecholamine stores are involved in the enhanced toxicity of *d*-amphetamine in aggregated (grouped) mice.[7, 8] This proposal was based in part on the finding that in aggregated mice the ability of *d*-amphetamine to deplete tissue stores of norepinephrine was enhanced. Endogenous catecholamines might also be involved in the enhanced toxicity of *d*-amphetamine in hyperthyroid mice. To test this proposal the effects of *d*-amphetamine on the norepinephrine concentrations in tissues of triiodothyronine-pretreated mice was examined. The results are summarized in Fig. 2 and Table 1.

There have been conflicting reports of changes in tissue catecholamine levels in hyperthyroid animals (e.g. Refs. 16–18). In the present study triiodothyronine injections did not alter tissue concentrations of norpinephrine. That is, there was no significant difference between the norepinephrine levels in euthyroid and hyperthyroid mice injected with saline (open and solid circles in Fig. 2 at zero dose of *d*-amphetamine). In euthyroid mice *d*-amphetamine produced a dose-dependent reduction

of the norepinephrine concentration in the brain, spleen, and heart; 10 mg *d*-amphetamine/kg caused a significant reduction ($P < 0.01$) in all three tissues. Although 1 mg/kg was not effective, 5 and 10 mg/kg produced a significantly greater depletion of norepinephrine ($P < 0.01$) in brain and spleen of hyperthyroid than of euthyroid mice. There was no significant difference in the norepinephrine-depleting action

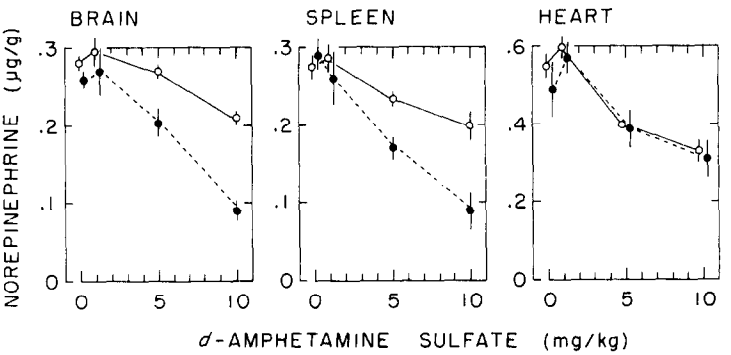

FIG. 2. Effect of *d*-amphetamine on tissue norepinephrine concentration of euthyroid and hyperthyroid mice. Each point represents the mean norepinephrine concentration in the tissues of euthyroid ○ and of hyperthyroid ● mice; the vertical line through each point represents one standard error of the mean. Each mean is based upon six determinations.

of *d*-amphetamine in the hearts of euthyroid and hyperthyroid mice. Despite the marked changes in brain, heart, and spleen norepinephrine stores, *d*-amphetamine caused no depletion of catecholamines in the adrenal glands of hyperthyroid mice (Table 1). It has previously been reported that *d*-amphetamine has no effect upon the adrenal catecholamine levels of individually caged or aggregated mice.[7]

TABLE 1. EFFECT OF *d*-AMPHETAMINE ON ADRENAL CATECHOLAMINES OF HYPERTHYROID MICE

	Dose (mg/kg)	Epinephrine	Norepinephrine
Saline		5·2 ± 0·29*	0·92 ± 0·26
d-Amphetamine	1	5·3 ± 0·25	0·74 ± 0·20
d-Amphetamine	5	5·0 ± 0·67	0·94 ± 0·22
d-Amphetamine	10	5·1 ± 0·32	0·78 ± 0·17

* Each value is based upon 5 determinations and represents the mean (± S.E.) of epinephrine and norepinephrine content expressed as micrograms per adrenal pair.

Effect of drug pretreatment on the toxity of d-*amphetamine in hyperthyroid mice*

The enhanced toxicity of *d*-amphetamine in aggregated mice is blocked by pretreatment with drugs that interfere with the actions or storage of endogenous catecholamines.[8] Since hyperthyroidism also potentiates the toxicity of *d*-amphetamine, it was decided to test the effects of these same drugs in hyperthyroid mice. The results are summarized in Table 2.

Chlorpromazine, a tranquilizing agent with mild adrenergic blocking properties, and phenoxybenzamine, an α-adrenergic blocking agent, both reduce the toxicity of *d*-amphetamine in aggregated mice.[8] These two agents quite effectively reduced the enhanced toxicity of *d*-amphetamine in hyperthyroid mice. Propranolol, a potent β-adrenergic blocking drug, although less effective, still produced a significant degree of protection.

In initial studies phenoxybenzamine was solubilized in 10% propylene glycol. Since propylene glycol has mild central depressant properties, there was a question of the relative importance of the actions of phenoxybenzamine and of propylene

TABLE 2. EFFECT OF DRUG PRETREATMENT ON THE TOXICITY OF *d*-AMPHETAMINE IN HYPERTHYROID MICE

	Dose (mg/kg)	Pretreatment time (hr)	No. of animals tested	Per cent mortality	
				2 hr	4 hr
Saline		0·5	24	96	96
Chlorpromazine	2	0·5	24	25*	29*
Phenoxybenzamine†	10	0·5	24	8*	17*
Phenoxybenzamine‡	10	0·5	24	13*	21*
Propranolol	5	0·5	24	21*	63*
Reserpine	0·2	24	24	100	100
Reserpine	1	24	30	94	94
α-Methyl-*m*-tyrosine§	500	24	28	79	93
α-Methyl-*p*-tyrosine	100	4	24	79	92

Mice were given i.p. injections of *l*-triiodothyronine (5 mg/kg) for 3 days; *d*-amphetamine (10 mg/kg) was injected ont he fourth day. The mice were pretreated at various times prior to the *d*-amphetamine injection.

* Represents those values that are significantly different from saline pretreatment at the 1% level.

† Solubilized with propylene glycol.

‡ Suspended in 0·5% methylcellulose.

§ The injection of this dose of α-methyl-*m*-tyrosine alone resulted in the deaths of 13 of 41 hyperthyroid mice.

glycol. As can be seen in Table 2, phenoxybenzamine was equally effective when dissolved in propylene glycol and when suspended in 0·5% methycellulose; the observed effect therefore is due to the phenoxybenzamine.

Unlike the protective effects in aggregated mice, pretreatment with reserpine and α-methyl-*m*-tyrosine did not block the enhanced toxicity to *d*-amphetamine in hyperthyroid mice. On the contrary α-methyl-*m*-tyrosine was itself toxic to hyperthyroid mice. For example, 3 of 25 hyperthyroid animals died during the 18-hr period following the injection of 100 mg α-methyl-*m*-tyrosine/kg, 13 of 41 died after 500 mg/kg. These doses of α-methyl-*m*-tyrosine caused no deaths in euthyroid mice. Pretreatment with α-methyl-*p*-tyrosine, a drug that reduces tissue norepinephrine stores but does not cause central excitation like the *meta* derivative (Moore, unpublished), was not toxic to the hyperthyroid mice, but it did not block the enhanced toxicity to *d*-amphetamine.

DISCUSSION

There have been numerous attempts to analyze the various factors involved in the toxicity of the amphetamines. The importance of such factors as body weight and ambient temperature are well recognized and must be taken into account when carrying out such studies. In an effort to obtain further insight into the mechanisms leading to death, we have previously examined chemical changes that occur in the tissues of aggregated mice after the administration of *d*-amphetamine. The importance of tissue norpinephrine stores for the actions of *d*-amphetamine in aggregated mice has been discussed elsewhere,[7, 8, 19] but the same considerations might also apply to the actions of *d*-amphetamine in hyperthyroid mice. That is, in conjunction with a stressful condition, in this case hyperthyroidism, *d*-amphetamine induces a greater than usual release of endogenous norepinephrine. The actions of the released norepinephrine, possibly potentiated by the high levels of circulating thyroid hormones, are important in the events leading to the death of hyperthyroid animals.

The results of the present investigation, although not unequivocal, do lend support to this hypothesis. Certainly there are similarities between the actions of *d*-amphetamine in aggregated and in hyperthyroid mice. The gross events leading to death are similar. In both groups marked excitation and increased motor activity are followed by the development of progressive depression ultimately leading to death. In both instances there is an enhanced depletion of catecholamines in brain but not in adrenal medulla. Chlorpromazine and phenoxybenzamine reduce the enhanced toxicity of *d*-amphetamine in both groups, and also in both groups *d*-amphetamine induces liver glycogen depletion and hypoglycemia.[19, 20]

Differences do exist, however, between the actions of *d*-amphetamine in aggregated and in hyperthyroid mice. The *d*-amphetamine-induced depletion of norepinephrine in heart was enhanced in aggregated mice but not in hyperthyroid mice. Reserpine and α-methyl-*m*-tyrosine pretreatment reduced the toxicity of *d*-amphetamine in aggregated mice: these agents were without protective effects in hyperthyroid mice. The protection of aggregated mice by these drugs was believed to result from their ability to deplete tissue norepinephrine stores.[8] If endogenous norepinephrine is important in the enhanced toxicity of *d*-amphetamine in hyperthyroid mice, then these same depleting drugs might be expected to exert a similar protection. The lack of protection by these two agents would appear to contradict the hypothesis that endogenous norepinephrine plays a role in the thyroid-enhanced toxicity to *d*-amphetamine.

In the present study, however, α-methyl-*m*-tyrosine was found to be lethal to hyperthyroid but not to euthyroid mice. In addition to its norepinephrine-depleting action, α-methyl-*m*-tyrosine causes an amphetamine-like central stimulation.[21] It is not surprising therefore that this substance, like *d*-amphetamine, shows an enhanced toxicity in hyperthyroid mice. Indeed, it is quite likely that the α-methyl-*m*-tyrosine toxicity, like that of *d*-amphetamine, is due in part to the actions of released catecholamines. Thus, the lack of protection by α-methyl-*m*-tyrosine to the lethality of *d*-amphetamine in hyperthyroid mice is quite probably due to the toxicity in these animals of α-methyl-*m*-tyrosine itself. The reasons for the lack of protection by reserpine are not clear. However, until more is known about the actions of these two agents in hyperthyroid animals, the above explanations must remain speculations.

Since chlorpromazine, phenoxybenzamine, and propranolol have adrenergic blocking properties in common, it has been assumed that they exert at least part

of their protection against amphetamine toxicity by virtue of their ability to block the actions of released endogenous norepinephrine. One might argue that this protective effect could result from something other than their adrenergic blocking properties. For example, phenoxybenzamine reduces the number of deaths resulting from traumatic shock.[22] However, even the antishock properties of phenoxybenzamine can now be ascribed to adrenergic blockade.[23]

The exaggerated release of norepinephine and the protective effect of adrenergic blocking drugs make it appear that norepinephrine does play a role in the enhanced toxicity of *d*-amphetamine in hyperthyroid mice. In addition, since the toxicities of other catecholamine-releasing drugs (α-methyl-*m*-tyrosine, ephedrine[11]) and catecholamine-potentiating drugs (monoamine oxidase inhibitors,[12] desmethylimipramine[14]) are also increased in hyperthyroid mice, it is quite probable that endogenous norepinephrine also plays an important role in the thyroid-enhanced toxicity of these drugs.

It should be noted that the doses of triiodothyronine used in the present study are excessive. We have now found that similar mortality curves for *d*-amphetamine can be obtained with doses of triiodothyronine which are 1/50 of those used in the present communication. With pretreatment with lower doses of triiodothyronine, *d*-amphetamine causes depletion of liver glycogen stores and marked hypoglycemia which could account for the death of the hyperthyroid mice.[20] Studies of the effects *d*-amphetamine on carbohydrate and electrolyte metabolism in hyperthyroid mice will be reported shortly.

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