Biochemical Pharmacology, 1966, Vol. 15, pp. 353–360. Pergamon Press Ltd., Printed in Great Britain.

AMPHETAMINE TOXICITY IN HYPERTHYROID MICE: EFFECTS ON BLOOD GLUCOSE AND LIVER GLYCOGEN*

KENNETH E. MOORE

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, U.S.A.

(*Received* 13 *September* 1965; *accepted* 25 *October* 1965)

Abstract—The effects of amphetamine and certain related drugs on mortality, blood glucose, and liver glycogen levels were compared in hyperthyroid and in euthyroid mice. Pretreatment with triiodothyronine markedly increased the mortality of mice injected with amphetamine, α-methyl-*m*-tyrosine, and ephedrine. In hyperthyroid but not in euthyroid mice these agents induced marked hypoglycemia and depletion of liver glycogen stores.

When tested under aggregated conditions, amphetamine caused similar effects on mortality, blood glucose, and liver glycogen stores in hypothyroid and euthyroid mice.

The mechanisms by which thyroid hormones influence the toxicity of amphetamine and related drugs are discussed in the light of these findings.

THE toxicity of amphetamine is increased by many stressful environmental factors.[1, 2] Some insight into the mechanisms by which these factors potentiate the toxicity of this drug has been obtained by measuring amphetamine-induced chemical changes in the tissues of stressed (aggregated or crowded) and nonstressed (isolated or individually caged) mice. In aggregated mice, but not in isolated mice, low doses of *d*-amphetamine depleted tissue norepinephrine stores,[3] reduced tissue glycogen stores, and produced hypoglycemia.[4] It was proposed that these changes are important events leading to the death of aggregated mice.

The toxicity of amphetamine is also enhanced in hyperthyroid mice.[5] Amphetamine induces similar behavioral effects (excitation followed by severe depression) and chemical effects (an exaggerated depletion of tissue norepinephrine stores) in both aggregated and in hyperthyroid mice.[3, 6]

In the present paper it will be shown that the toxicity of amphetamine and certain amphetamine-like drugs is markedly enhanced in hyperthyroid mice, and the death of these animals, like that of the aggregated mice, is preceded by depletion of liver glycogen and hypoglycemia. The extent of the hypoglycemia is such that it undoubtedly contributes to the death of the hyperthyroid mice.

METHODS

Male albino mice (Charles River Mouse Farms) weighing 24–30 g were used throughout this study. The effects of amphetamine were examined in (i) hyper- and euthyroid mice caged individually and in (ii) hypo- and euthyroid mice under aggregated conditions (four mice per cage).

* Supported by Grant AM-06275 from the National Institute of Arthritis and Metabolic Diseases. A preliminary report of this work appeared in *Fed. Proc.* **24**, 518 (1965).

In the initial studies [(i) above] mice were injected with triiodothyronine (hyperthyroid mice) or with alkaline saline (control, euthyroid mice) on three consecutive days. On the fourth day they were injected with saline or drugs and placed individually into small (9 × 9 × 9 cm) wire mesh cages. A variation of this experimental procedure is described in the legend to Table 1.

In the second study [(ii) above] animals were made hypothyroid by replacing their drinking water with 1% potassium perchlorate for 2 weeks. At the end of this time, the hypothyroid mice and their euthyroid controls were injected with *d*-amphetamine or saline and placed four per cage in the small wire mesh cages. The results of this experiment are depicted in Table 3; this is the only study that involved aggregated mice.

During pretreatment the animals were housed in large cages in groups of twenty-four. Food but not water was removed from these cages 4 hr before the animals were injected with saline or drugs. All experiments were performed in a room where the circulating air was maintained at 24 ± 0·5°. Mortality data were obtained from the number of mice dying during the 4-hr period after injection. Blood glucose and liver glycogen determinations were made in animals that were sacrificed 1·5–2 hr after injection by decapitation. Blood was collected in heparinized beakers and small samples of liver quickly removed, weighed, and digested in boiling 30% KOH. Glycogen was determined by the anthrone method as described by Hassid and Abraham,[7] and glucose was determined in 0·2-ml aliquots of blood by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N.J.).

Total-body oxygen consumption was determined at various times after the injection of triiodothyronine, with a volume meter model 160 (Med-Science Electronics, St. Louis, Mo.). Food was removed from the mice 4–6 hr before these measurements.

Drug solutions were adjusted so that the prescribed dose was injected i.p. in a volume of 1 ml/100 g body weight. *d*-Amphetamine sulfate, ephedrine sulfate, and *dl*-α-methyl-*m*-tyrosine monohydrate (αMmT)* were dissolved in water. α-Methyl-*l*-dihydroxyphenlalanine (αMDOPA) was suspended in 0·5% methylcellulose. *l*-Triiodothyronine (*l*-T_3) was dissolved in a small volume of 0·1 N sodium hydroxide, the pH adjusted to 10 and the solution made to volume with water. Alkaline saline was prepared by adding a similar amount of 0·1 N sodium hydroxide to 0·9% NaCl and adjusting the resulting solution to pH 10. The doses of all drugs are expressed as their respective salts.

RESULTS

Mortality of hyperthyroid mice to d-*amphetamine*

In a previous study[6] it was reported that pretreatment of mice for 3 days with 5 mg triiodothyronine/kg markedly increased the lethality to *d*-amphetamine. Since daily injections of 0·1–5·0 mg triiodothyronine/kg result in a similar per cent mortality in mice receiving 10 mg *d*-amphetamine/kg (Fig. 1), an excessive quantity of the hormone was obviously administered in the earlier study. Even 0·01 of the dose used in the previous study markedly potentiated the lethality of *d*-amphetamine (i.e. 70% mortality with 0·05 mg *l*-triiodothyronine/kg). The per cent mortality of all

* The abbreviations used are: αMmT, *dl*-α-methyl-*m*-tyrosine monohydrate; αMDOPA, α-methyl-*l*-dihydroxyphenylalanine; *l*-T_3, *l*-triiodothyronine.

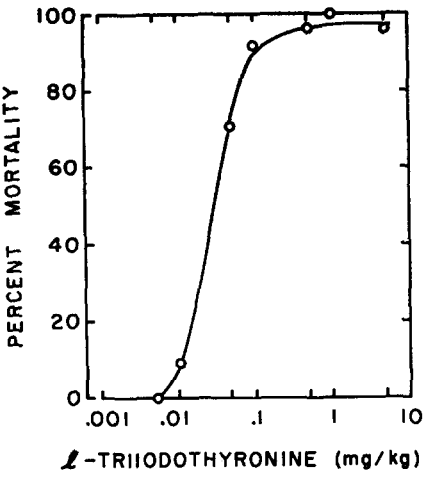

FIG. 1. Lethality of *d*-amphetamine in mice pretreated with triiodothyronine. Each point represents the per cent mortality to *d*-amphetamine (10 mg/kg) as determined in 24 mice pretreated for 3 days with the doses of triiodothyronine indicated.

mice pretreated with triiodothyronine was dependent upon the dose of *d*-amphetamine. In the present study only the effects of 10 mg *d*-amphetamine/kg are reported.

Effects of d-*amphetamine on blood glucose and liver glycogen of hyperthyroid mice*

Excitement and increased motor activity were observed in both euthyroid and hyperthyroid mice several minutes after the injection of *d*-amphetamine. However, 1–2 hr after injection, the excitement in hyperthyroid mice was replaced by a period of depression or exhaustion which ultimately led to death. Blood glucose and liver glycogen levels were determined 1·5–2 hr after the injection of *d*-amphetamine, at a time when the euthyroid mice were excited but the hyperthyroid mice depressed.

When compared with controls, triiodothyronine pretreatment caused a reduction in the blood glucose and liver glycogen concentrations. In control or euthyroid mice, *d*-amphetamine produced no significant effect upon the levels of blood glucose or liver glycogen; in hyperthyroid mice *d*-amphetamine caused marked hypoglycemia and almost complete depletion of liver glycogen. As seen in Fig. 2, there was little difference between the effects at the two doses of triiodothyronine tested (0·1 and 5·0 mg/kg).

Time course of triiodothyronine actions

An effort was made to correlate the onset of the enhanced toxicity of *d*-amphetamine with the onset of some action of triiodothyronine. The time courses of the effects of triiodothyronine on whole-body oxygen consumption, on blood glucose and liver glycogen concentrations, and on *d*-amphetamine toxicity are depicted in Table 1. A high dose of triiodothyronine (5·0 mg/kg) was used in this study in order to determine the earliest onset of the hormone's effects.

Sixteen hours after the injection of triiodothyronine, the oxygen consumption of the mice was significantly higher than controls ($P < 0{\cdot}05$). It remained at this higher level throughout the treatment period. The blood glucose and liver glycogen levels fell

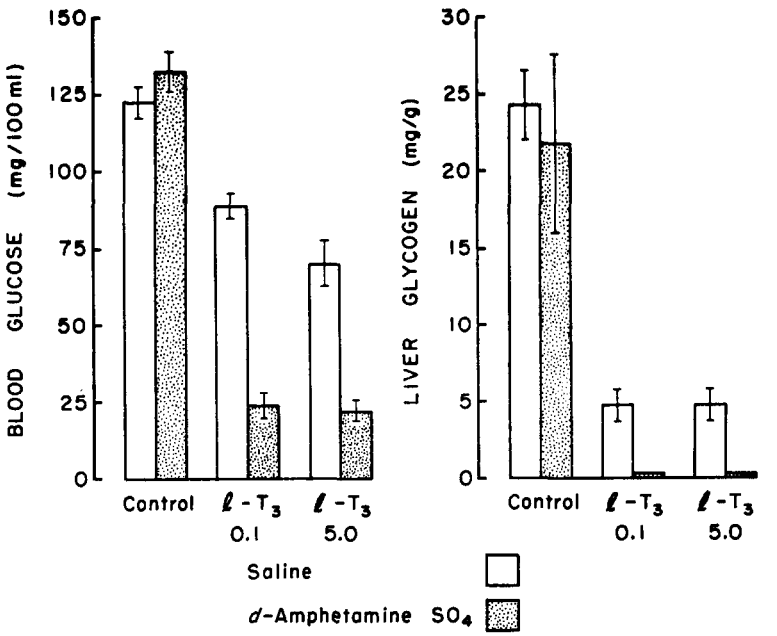

FIG. 2. Effects of *d*-amphetamine (10 mg/kg) on blood glucose and liver glycogen levels of control and triiodothyronine-pretreated mice. The pretreatment schedule was as described in Methods. Control represents those mice pretreated with alkaline saline; *l*-T_3 0·1 and 5·0 represent mice pretreated with 0·1 and 5·0 mg/kg *l*-triiodothyronine.

The height of each bar represents the mean obtained from eight animals and the line projected upon it the standard error of that mean.

progressively during the first 24 hr and then remained at these low levels throughout the rest of the treatment period. After 8 hr all glucose and glycogen values from triiodothyronine-treated mice were significantly less than saline-treated controls ($P < 0{\cdot}01$). When *d*-amphetamine was injected concurrently with, or 2, 4, or 8 hr

TABLE 1. TIME COURSE OF THE EFFECTS OF TRIIODOTHYRONINE ON OXYGEN CONSUMPTION, BLOOD GLUCOSE, AND LIVER GLYCOGEN LEVELS OF SALINE-TREATED MICE AND ON THE MORTALITY OF *d*-AMPHETAMINE-TREATED MICE

Hours after *l*-T_3*	O_2 consumption (ml O_2/kg/min)	Blood glucose (mg/100 ml)	Liver glycogen (mg/g)	Mortality to *d*-amphetamine (%)
0	56·5 ± 2·3	149 ± 3·3	22·92 ± 3·05	0
8	56·0 ± 2·8	114 ± 3·3	10·51 ± 2·09	0
16	70·8 ± 4·7	110 ± 6·3	6·69 ± 1·52	18
24	66·5 ± 6·6	83 ± 5·0	1·14 ± 0·36	31
48	66·8 ± 3·3	80 ± 3·0	1·72 ± 0·49	69
72	73·6 ± 6·7	74 ± 2·2	2·53 ± 0·74	95

* Hours after *l*-T_3 was taken from the first injection (0 hr) until the injection of saline or *d*-amphetamine. Up to 24 hr, mice received one *l*-T_3 injection. Mice in the 48 hr study received two (at 0 and 24 hr), and in the 72-hr study three (at 0, 24, and 48 hr) injections of *l*-T_3.

Percent mortality to *d*-amphetamine (10 mg/kg) was calculated from the number of deaths in 16–56 treated mice. All other values represent the mean ± standard error: for oxygen consumption 4–8 separate determinations, for blood glucose and liver glycogen 8 determinations.

after the injection of triiodothyronine, no deaths occurred. It was not until after an injection interval of 16 hr that *d*-amphetamine caused any deaths; the per cent mortality to *d*-amphetamine then increased progressively throughout the treatment period.

Thus, at best, only a rough correlation exists between the onset of the triiodothyronine-enhanced lethality to *d*-amphetamine and the onset of the triiodothyronine-induced reduction of blood glucose and liver glycogen stores.

Effects of some catecholamine-releasing drugs in hyperthyroid mice

The toxicity of αMmT, ephedrine, and αMDOPA in euthyroid and hyperthyroid mice are compared in Table 2. These agents, like amphetamine, are capable of releasing catecholamines from endogenous stores. Doses of ephedrine and αMmT which were without gross toxic effects to euthyroid mice were lethal to some of the hyperthyroid mice. Marked hypoglycemia and depletion of liver glycogen also developed in these animals. αMDOPA was not lethal to the hyperthyroid mice, and it did not significantly reduce the blood glucose or liver glycogen concentrations in these animals. None of these agents had a significant effect on the blood glucose levels or glycogen stores of euthyroid mice.

TABLE 2. EFFECTS OF EPHEDRINE, αMmT AND αMDOPA ON BLOOD GLUCOSE, LIVER GLYCOGEN, AND MORTALITY OF EUTHYROID AND OF HYPERTHYROID MICE

Pretreatment	Treatment	No. dead / No. tested	Blood glucose (mg/100 ml)	Liver glycogen (mg/g)
Alkaline saline (euthyroid)	Saline	0/24	135·8 ± 8·3	20·6 ± 4·7
	Ephedrine	0/26	148·3 ± 10·0	37·1 ± 5·7
	αMmT	0/26	140·5 ± 5·2	35·9 ± 3·0
	αMDOPA	0/26	138·7 ± 7·9	27·6 ± 3·8
Triiodothyronine (hyperthyroid)	Saline	0/24	88·7 ± 4·2	4·67 ± 0·99
	Ephedrine	18/26*	19·6 ± 2·4*	0·12 ± 0·01*
	αMmT	8/26*	36·5 ± 8·2*	0·12 ± 0·03*
	αMDOPA	0/26	79·8 ± 4·9	1·97 ± 0·72

The pretreatment schedule consisted of injections of alkaline saline or triiodothyronine, for three consecutive days; on the fourth day saline, ephedrine sulfate (50 mg/kg), αMmT (500 mg/kg), or αMDOPA (500 mg/kg) was injected. The blood glucose and liver glycogen values represent the mean ± standard error of that mean as determined from 8 analyses.

* Those values that are significantly different ($P < 0.01$) from saline-injected mice.

Effects of d-*amphetamine in hypothyroid mice*

Since hyperthyroid mice are markedly sensitive to the toxicity of *d*-amphetamine, it was decided to examine the sensitivity of hypothyroid mice to this agent. Mice were made hypothyroid by substituting a 1% aqueous solution of potassium perchlorate for drinking water for 2 weeks. After this period the oxygen consumption of the perchlorate-treated mice was significantly lower ($P < 0.01$) than that of control euthyroid mice (Table 3). The blood glucose was slightly but not significantly greater than that of controls; the liver glycogen concentration was significantly greater ($P < 0.01$).

TABLE 3. EFFECTS OF *d*-AMPHETAMINE IN EUTHYROID AND HYPOTHYROID MICE UNDER AGGREGATED CONDITIONS

	Euthyroid	Hypothyroid
Saline		
Oxygen consumption	56·5 ± 2·3 (6)	47·1 ± 1·6 (10)
Blood glucose	135·8 ± 8·3 (8)	153·0 ± 7·2 (8)
Liver glycogen	20·6 ± 4·7 (8)	48·2 ± 5·9 (8)
d-Amphetamine		
No. dead	27/40	27/40
No. tested		
Blood glucose	17·3 ± 2·1 (8)	21·0 ± 4·8 (8)
Liver glycogen	0·13 ± 0·02 (8)	0·15 ± 0·02 (8)

Values represent means ± standard errors; the numbers in parentheses indicate the number of determinations. Oxygen consumption is expressed as ml oxygen/kg body wt./min, glucose as mg/100 ml of blood, and glycogen as mg/g wet wt. of liver. Animals were injected with Saline or *d*-Amphetamine (20 mg/kg) and placed in small wire mesh cages (four mice/per cage) for a period of 2 hr for blood glucose and liver glycogen determinations and for 4 hr in the mortality studies.

The toxicity of *d*-amphetamine was tested in euthyroid and in hypothyroid mice under aggregated conditions. That is, after injection of 20 mg *d*-amphetamine/kg, mice were placed four per cage in small (9 × 9 × 9 cm) wire mesh cages (see Ref. 3 for further details). When tested in this manner there was no significant difference in the per cent mortality of euthyroid and of hypothyroid mice. Also, in both groups *d*-amphetamine induced a marked hypoglycemia and almost complete depletion of liver glycogen. Thus, under aggregated conditions, hypothyroid and euthyroid mice are equally sensitive to the toxicity of *d*-amphetamine.

DISCUSSION

The responses to some drugs can be influenced by a number of environmental factors which might affect drug actions by altering the activity of the autonomic nervous system or by producing abnormal endocrine states. During the past several years we have attempted to study this problem by examining the factors that influence the actions of amphetamine.

The toxicity of amphetamine is enhanced in both aggregated and hyperthyroid mice. Indeed, many of the actions of amphetamine are similar in these two groups.[3, 4, 6] In both, an initial period of excitement and increased motor activity is followed in 1–2 hr by a depressed or exhausted state which ultimately leads to death. In both aggregated and hyperthyroid mice amphetamine causes marked depletion of norepinephrine from tissue depots, and in both groups the lethality of amphetamine is blocked by pretreatment with phenoxybenzamine and chlorpromazine. In aggregated mice the development of the depressed state is accompanied by hypoglycemia and depletion of glycogen from the liver.[4] In the present report *d*-amphetamine was shown to exert the same effect in hyperthyroid mice.

In studies with both aggregated and hyperthyroid mice only those animals depressed at the time of sacrifice were hypoglycemic. The animals that did not exhibit depression did not have significantly lower blood glucose levels. This indicates that, regardless of the cause of the depression, its onset coincides with and may be causally

related to the hypoglycemia. However, the role of hypoglycemia in the exaggerated toxicity of amphetamine has not been clearly defined; results of experiments to test this role have been equivocal. In unpublished experiments we have found that insulin-induced hypoglycemia increased the per cent mortality of aggregated mice to amphetamine. On the other hand, when liver glycogen and blood glucose levels were lowered by starvation, there was a reduction in the number of aggregated mice dying. This latter effect probably results from the reduced body weight of the starved animals.[8] Attempts at preventing the death of aggregated mice by giving repeated injections of glucose have thus far proven unsuccessful. Although the hypoglycemia is probably not the primary cause of death of the amphetamine-treated aggregated or hyperthyroid mice, it certainly must contribute to their death, since the values for blood glucose in these animals (less than 25 mg/100 ml) approach those in mice dying as a result of insulin hypoglycemia (Moore, unpublished).

In addition to amphetamine, the toxicity of several other drugs is increased in hyperthyroid animals. These drugs have in common the ability to release catecholamines from endogenous stores or to potentiate the actions of the catecholamines (e.g. monoamine oxidase inhibitors,[9] ephedrine,[5] imipramine[10]). However, the ability of a drug to release catecholamines from endogenous stores does not in itself ensure that its toxicity will be enhanced in hyperthyroidism. It appears that, in addition to its ability to release or potentiate the actions of the catecholamines, a drug must have stimulating action on the central nervous system. For example, ephedrine, αMmT, and amphetamine all have central stimulant actions;[11] all are more toxic to hyperthyroid mice than to euthyroid mice. On the other hand, αMDOPA releases catecholamines but has central sedative actions;[12] its toxicity is not increased in hyperthyroid mice. It appears then that central nervous stimulation and peripheral release of catecholamines may combine to exert a deleterious action which ultimately leads to depletion of tissue glycogen stores, hypoglycemia, and death.

Askew[13] reported that thyroxine pretreatment potentiated the toxicity of amphetamine in aggregated mice; it is now apparent that the same is true for individually caged mice.[5, 6] The results of the present study indicate that the thyroid gland probably plays little role in the studies with aggregated mice. First, even with massive doses of triiodothyronine (5 mg/kg), there was a lag of approximately 16 hr between the administration of the hormone and the onset of the increased toxicity of amphetamine. Second, when they were tested in aggregated conditions, there was no significant difference between the mortality of hypothyroid and euthyroid mice.

The primary deleterious effect of amphetamine and related drugs in hyperthyroid mice is not known. Although several of the actions of triiodothyronine roughly correlate with the onset of the amphetamine toxicity, it is difficult to ascribe a "primary" effect to one. The delayed onset of the triiodothyronine effect does suggest that the phenomenon is linked to a metabolic modification caused by the hormone. Those effects measured (increased oxygen consumption, lowered glycogen and glucose levels) could play a role, but the ability of thyroid to potentiate the responses of the catecholamines is probably the most important factor in initiating the exaggerated response to amphetamine. Bray and Goodman[14] have recently shown that the latent period after triiodothyronine is shortest for those effects arising from increased sensitivity to the catecholamines. The role of endogenous catecholamines in the thyroid-enhanced toxicity of amphetamine has been discussed by the author.[6]

REFERENCES

1. M. R. A. CHANCE, *J. Pharmac. exp. Ther.* **87**, 214 (1946).
2. B. WEISS, V. G. LATIES and F. L. BLANTON, *J. Pharmac. exp. Ther.* **132**, 366 (1961).
3. K. E. MOORE, *J. Pharmac. exp. Ther.* **142**, 6 (1963).
4. K. E. MOORE, L. C. SAWDY and S. R. SHAUL, *Biochem. Pharmac.* **14**, 197 (1965).
5. B. N. HALPERN, C. DRUDI-BARACCO and D. BESSIRARD, *Nature Lond.* **204**, 387 (1964).
6. K. E. MOORE, *Biochem. Pharmac.* **14**, 1831 (1965).
7. W. Z. HASSID and S. ABRAHAM, *Methods in Enzymology* **3**, 37 (1956).
8. G. B. FINK and R. E. LARSON, *J. Pharmac. exp. Ther.* **137**, 361 (1962).
9. R. N. CARRIER and P. V. BUDAY, *Nature Lond.* **191**, 1107 (1961).
10. A. J. PRANGE, Jr. and M. A. LIPTON, *Nature Lond.* **196**, 588 (1962).
11. P. L. CARLTON, *Nature Lond.* **200**, 586 (1963).
12. J. M. VAN ROSSUM and J. A. TH. M. HURKMANS, *J. Pharm. Pharmac.* **15**, 493 (1963).
13. B. M. ASKEW, *Br. J. Pharmac.* **19**, 245 (1962).
14. G. A. BRAY and H. M. GOODMAN, *Endocrinology* **76**, 323 (1965).