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EFFECTS OF D-AMPHETAMINE ON BLOOD GLUCOSE AND TISSUE GLYCOGEN LEVELS OF ISOLATED AND AGGREGATED MICE*

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Abstract—Blood glucose and tissue glycogen levels were determined in isolated and in aggregated (4/cage) mice at various times after the injection of *d*-amphetamine sulfate (10 mg/kg). In individually caged mice amphetamine caused excitement and increased motor activity but did not cause death. In aggregated mice the amphetamine-induced excitement and motor activity were markedly enhanced but were followed in 1–2 hr by a state of depression. Approximately 25% of the aggregated animals died in this depressed state.

d-Amphetamine had little effect on blood glucose or tissue glycogen levels of isolated mice. In aggregated mice *d*-amphetamine caused a marked reduction in the glycogen stores in the liver, skeletal muscle, and brain. This was true for both the depressed and nondepressed animals. Only depressed animals had significantly lower blood glucose levels. Those drugs (phenoxybenzamine, chlorpromazine, reserpine, and α-methyl-*m*-tyrosine) that blocked the depression and lethality of *d*-amphetamine also blocked the hypoglycemia and liver glycogen depletion.

Since the onset of the depressed state occurred contemporaneously with the development of the hypoglycemia, these phenomena may be causally related and may be important events leading to the death of aggregated mice.

IN 1946 Chance[1] reported that aggregation (the grouping of mice in small cages) potentiated the toxicity of amphetamine. Although studied by many other workers, the mechanisms by which aggregation enhances the toxicity of amphetamine have not been fully elucidated. Some of the factors that influence the aggregation effect include: temperature,[2–4] motor activity,[5, 6] and the hormonal state of the animals.[7, 8] The importance of these factors is now recognized and must be taken into account when determining the toxicity of amphetamine. However, correlations between these factors and toxicity do not necessarily establish causation, and they are inadequate to explain the mechanism of death.

Some insight into the events leading to the death of aggregated mice might be obtained by examining amphetamine-induced biochemical changes. In the past few years we have examined various chemical changes occurring in the tissues of aggregated mice after the injection of *d*-amphetamine. We have reported that aggregation increases the ability of *d*-amphetamine to release norepinephrine from endogenous

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stores.[9] Procedures that interfere with the actions or release of endogenous norepinephrine block the effects of aggregation. As a result of these studies, it was proposed that the release of norepinephrine from endogenous stores is an important factor in the increased toxicity to *d*-amphetamine.[10] However, the mechanisms by which norepinephrine causes its deleterious effects are not known.

There are several possible ways in which norepinephrine might exert untoward effects. The massive release of this amine, either by interfering with heat loss (through peripheral vascular effects) or by increasing heat production (by a calorigenic action), could account for the marked hyperthermia. The effects of norepinephrine on the cardiovascular system might also be considered. The ability of norepinephrine to mobilize energy fuels and to regulate carbohydrate metabolism could also be an important consideration.

This report is concerned with this last aspect and describes the effects of *d*-amphetamine on the blood glucose and tissue glycogen levels in isolated and in aggregated mice. As will become evident, *d*-amphetamine induces marked hypoglycemia in aggregated but not in isolated mice. The degree of hypoglycemia is such that it could well account for the death of the aggregated mice.

METHODS

All experiments were carried out with male albino mice (Charles River Mouse Farm) weighing 24–30 g. They were housed in large wire-mesh cages in groups of 30 and had free access to food and water. Four hours before the start of each experiment food but not water was removed from their cages. The experiments consisted of injecting mice with saline or *d*-amphetamine and placing them in small (9-cm square) wire-mesh cages, with 4 mice per cage in the "aggregated" series and 1 per cage in the "isolated" series. Room temperature was maintained at 24 $\pm$ 0·5°.

The mice remained in the small cages for varying periods up to 2 hr, during which time they received neither food nor water; 30, 60, and 120 min after the injection of saline or *d*-amphetamine, mice from both the isolated and the aggregated series were decapitated so that their heads fell into a dry-ice alcohol mixture. Blood was collected in heparinized beakers. Samples of skeletal muscle and liver were quickly removed, weighed, and digested in boiling 30% KOH. Pieces of frozen brain were chiseled out of the skull, weighed in a $-$ 20° cold room, and then digested in boiling 30% KOH.

Glycogen was determined by the anthrone method as described by Hassid and Abraham.[11] Briefly, glycogen from all tissues was precipitated with alcohol and washed twice. In addition, glycogen from brain was washed twice with hot methanol: chloroform (4:1 v/v). The glycogen was dissolved in water and analyzed colorimetrically after the addition of anthrone reagent. Glucose was determined in 0·2-ml aliquots of blood by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N.J.).

All drug solutions were prepared immediately prior to their use and were adjusted so that the prescribed dose was injected in a volume of 1 ml/100 g body weight. The salts, in which the doses of all drugs are expressed, and the solvents used to dissolve them were as follows: *d*-amphetamine sulfate, chlorpromazine HCl, ergotamine tartrate (Gynergen ampoules), α-methyl-*m*-tyrosine and Insulin Injection, U.S.P., all dissolved in or diluted with water. Phenoxybenzamine HCl was dissolved in propylene glycol (final concentration of 10%) and diluted to volume with water. Crystalline

reserpine was dissolved in glacial acetic acid, partially neutralized and, diluted to volume with water. Ergotamine and insulin were injected s.c.; all other drugs were injected i.p.

RESULTS

Non-pretreated animals. Under the conditions of these experiments, the LD_{50} (and 95% fiducial limits) for *d*-amphetamine in isolated mice was 98 (90–107) mg/kg; for aggregated mice it was 20 (14·5 –27·8) mg/kg. In all studies reported in the present paper, *d*-amphetamine was injected at a dose of 10 mg/kg, a dose at which none of the isolated (and approximately 25% of the aggregated) mice died. In the individually caged mice the injection of this dose of *d*-amphetamine caused excitement and increased motor activity; it did not cause death. In aggregated mice the *d*-amphetamine-induced excitement and motor activity were markedly enhanced. However, 1–2 hr after injection, some of the aggregated animals lay quietly in the cages in an exhausted or depressed state. It is during this depressed state that many of the aggregated mice die. (Approximately 25% of these mice die within 4 hr after the injection of 10 mg *d*-amphetamine/kg.)

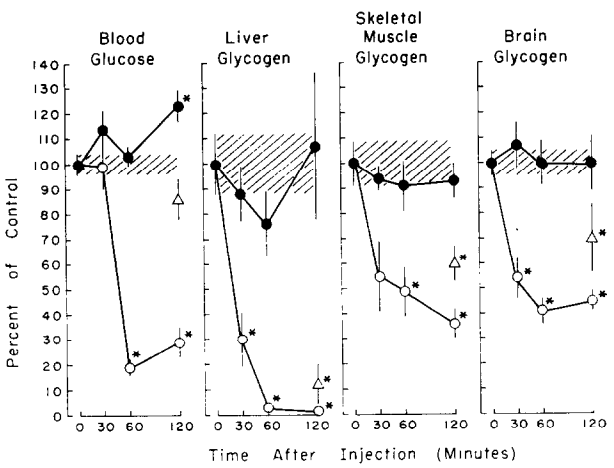

FIG. 1. Effects of *d*-amphetamine on the blood glucose and tissue glycogen levels of isolated and aggregated mice. All values are plotted as percentages of mean values obtained in saline-treated aggregated mice; the shaded areas represent the standard errors of the means of these control values. All mice received 10 mg *d*-amphetamine/kg and were then placed in cages singly (●) or as groups (4/cage) (○ and △). (○), Animals that appeared depressed at the time of sacrifice. (△), Animals that showed no depression; each point represents the mean obtained from 8 animals; the vertical line through the point represents the standard error of this mean. *, Values that are significantly different from control values ($P < 0{\cdot}01$).

In Fig. 1 the effects of *d*-amphetamine on the blood glucose and tissue glycogen levels of isolated and aggregated mice are plotted as a percentage of control values. The control values were obtained from mice injected with saline and held for 2 hr in small cages either individually or in groups of 4. There was no difference between the values obtained from isolated and aggregated saline-treated mice. The control values (mean $\pm$ S.E.) were: 123 $\pm$ 5 mg/100 ml for blood glucose; 20·2 $\pm$ 2·5 mg/g for

liver glycogen; 2·70 ± 0·24 mg/g for skeletal muscle glycogen; and 0·54 ± 0·028 mg/g for brain glycogen.

In the isolated mice, *d*-amphetamine produced no significant changes in blood glucose or tissue glycogen levels except for a slight but significant elevation of blood glucose at 120 min. In crowded mice, on the other hand, *d*-amphetamine induced a marked reduction in the blood glucose level after 60 min. This effect was preceded by an appreciable reduction in the liver glycogen content which was first observed at 30 min. Significant but less marked reductions in skeletal muscle and brain glycogen were observed. The 60- and 120-min results indicated by ○ were obtained from animals which were depressed at the time of sacrifice. The 120-min values from those animals that were not overtly depressed at the time of sacrifice are represented by △. The amount of tissue glycogen in these nondepressed animals was significantly reduced; the blood glucose value, however, was not significantly different ($P > 0·10$) from that of control. Thus, *d*-amphetamine had little effect on blood glucose or tissue glycogen levels in isolated mice. In aggregated mice, although both the depressed and

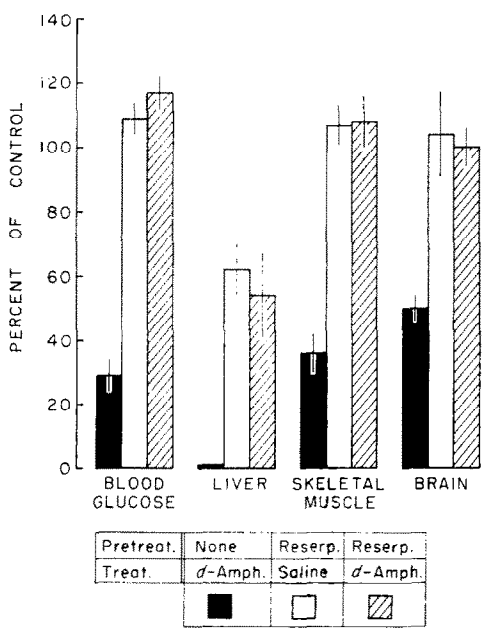

FIG. 2. Effects of reserpine pretreatment on blood glucose and tissue glycogen levels of amphetamine- and saline-treated aggregated mice. All values are plotted as a percentage of saline-treated aggregated mice. The height of each bar represents the mean obtained from 8 animals, and the line projected upon it the standard error of that mean. Mice were injected with reserpine (0·2 mg/kg) 24 hr before injection of saline or *d*-amphetamine (10 mg/kg). All animals were sacrificed 120 min after the second injection.

the nondepressed group had significantly reduced glycogen stores, only the depressed animals showed a significant reduction in blood glucose.

Drug-pretreated animals. It has been reported previously[10, 12–15] that pretreatment with drugs that modify the actions or release of endogenous norepinephrine reduces the toxicity of *d*-amphetamine in aggregated mice. If hypoglycemia is an important

cause of death, those drugs that reduce the toxicity of *d*-amphetamine in aggregated mice should also block the development of hypoglycemia. Accordingly, we have examined the effects of these drugs on the *d*-amphetamine-induced hypoglycemia and glycogen depletion in aggregated mice.

Pretreatment with reserpine was shown previously,[10, 12] to reduce the number of aggregated mice dying after an injection of *d*-amphetamine. In the present study, when mice were pretreated with reserpine (0·2 mg/kg) 24 hr prior to injections of *d*-amphetamine (10 mg/kg), the depression and lethality in the aggregated mice were completely prevented. Figure 2 shows the effects of reserpine pretreatment on their blood glucose and tissue glycogen content. In saline-injected mice, reserpine pretreatment caused a slight reduction in liver glycogen stores but had no effect on the glycogen content of other tissues and did not influence the blood glucose. In reserpinized mice injected with *d*-amphetamine, the glycogen and blood glucose values were not significantly different from those of the saline-treated animals. Thus, reserpine pretreatment completely blocked the amphetamine-induced reductions of blood glucose and tissue glycogen in aggregated mice.

Chlorpromazine, phenoxybenzamine, and α-methyl-*m*-tyrosine have been reported previously to reduce the toxicity of *d*-amphetamine in aggregated mice.[10, 14, 15] In a

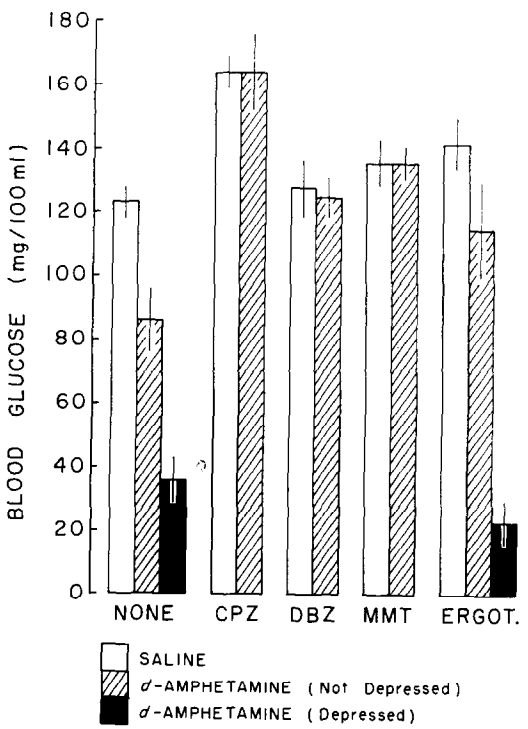

FIG. 3. Effects of drug pretreatment on the blood glucose levels of saline- and *d*-amphetamine-treated aggregated mice. The height of each bar represents the mean obtained from 8 animals, and the line projected upon it the standard error of that mean. The pretreatment dosages were: CPZ = chlorpromazine, 2 mg/kg; DBZ = phenoxybenzamine (Dibenzyline), 10 mg/kg; MMT = α-methyl-*m*-tyrosine, 500 mg/kg; ERGOT. = ergotamine, 2 mg/kg. MMT was injected 24 hr and all other drugs 30 min before the saline or *d*-amphetamine injections. All animals were sacrificed 120 min after the second injection.

typical experiment under the conditions used in the present study, the number of aggregated mice dying from an injection of *d*-amphetamine (10 mg/kg) was: 3/16 for non-pretreated; 0/16 for those treated with α-methyl-*m*-tyrosine, chlorpromazine, and phenoxybenzamine; and 7/16 for ergotamine-pretreated mice. Chlorpromazine, phenoxybenzamine, and α-methyl-*m*-tyrosine reduce the toxicity, whereas ergotamine has no protective action and actually may enhance the toxicity of *d*-amphetamine. Figure 3 depicts the effects of these same drugs on the blood glucose levels of aggregated mice. Except for chlorpromazine, which produced an increase, pretreatment with these drugs had little effect on the blood glucose of control or saline-treated mice. Chlorpromazine, phenoxybenzamine, and α-methyl-*m*-tyrosine, the drugs that prevented the development of depression and death in *d*-amphetamine-treated aggregated mice, also prevented the hypoglycemia. On the other hand, those ergotamine-pretreated mice that became depressed exhibited marked hypoglycemia.

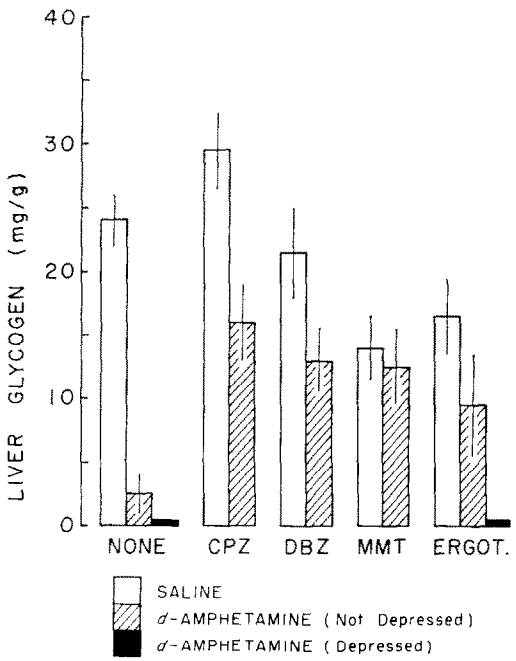

FIG. 4. Effects of drug pretreatment on the liver glycogen content of saline- and *d*-amphetamine-treated aggregated mice. See legend of Fig. 3 for further details.

Similar effects were seen when the liver glycogen content of these animals was determined (Fig. 4). Chlorpromazine, phenoxybenzamine, and α-methyl-*m*-tyrosine each prevented the depletion of liver glycogen. Thus, those drugs that prevent the development of the exhausted or depressed state also prevent the development of hypoglycemia and liver glycogen depletion.

DISCUSSION

The many factors that influence the toxicity of *d*-amphetamine in isolated and grouped mice have been well documented.[1-4, 6, 9] From an examination of the slopes

of the dose–mortality curves and from gross observations of behavior, it is obvious that the cause of death in the two groups is different. Isolated mice usually die after convulsions some 5–30 min after the injection of *d*-amphetamine. In aggregated mice death occurs 1–4 hr after the injection of a much lower dose of *d*-amphetamine. After a 1–2 hr period of excitement, motor activity, and occasional fighting, some animals lapse into an exhausted or depressed state. Generally the animals that become depressed eventually die; those that do not become depressed survive. The present work was designed to examine whether biochemical changes in the tissues of the aggregated mice could account for these effects.

As a result of the present experiments, it appears that the depletion of liver glycogen and the ensuing hypoglycemia can at least partially account for the depressed state and eventual death of the aggregated mice. This statement is based on the following observations:

1. The depressed state occurs only in aggregated mice; only aggregated mice develop hypoglycemia.

2. Only those aggregated mice that show signs of depression are hypoglycemic.

3. The onset of the depression occurs contemporaneously with the onset of hypoglycemia.

4. Those drugs that prevent the onset of the depressed state and death also prevent the development of the hypoglycemia.

5. When the blood glucose concentration of nonaggregated mice was lowered with insulin injections to levels equivalent to those in the depressed aggregated mice, they became progressively more depressed, exhibited labored breathing, and occasionally convulsed prior to death. This sequence closely resembled that seen in the aggregated mice (unpublished results).

Many stressful situations can produce an initial hyperglycemic response, which is followed by a secondary period of hypoglycemia and depleted tissue glycogen stores.[16] The combined effects of *d*-amphetamine and crowding obviously serve as a severe stress and produce the typical effects except that the initial hyperglycemia was not observed. It may have been missed because blood samples were not taken frequently enough or, since food was removed for several hours prior to the start of the experiment, it may not have occurred. It has been previously reported that the initial hyperglycemic phase is not observed in stressed rats which have been starved.[17]

A similar state of depression, tissue glycogen depletion, and hypoglycemia develops during the latter stages of various types of experimental shock.[18, 19] It has been postulated that these changes are secondary to cardiovascular depression, ischemia, and tissue anoxia. We have noted that it is often difficult to obtain blood from the depressed mice, indicating perhaps that the blood pressure is reduced to shock levels. Further studies are needed to establish this inference. It is, however, quite conceivable that a shock-like syndrome develops in the aggregated animals which then accounts for the observed effects on the carbohydrate stores. It is interesting to note that some of the drugs that reduce the lethality of *d*-amphetamine in aggregated mice (chlorpromazine and phenoxybenzamine) are also effective in preventing death in many experimental shock situations. Indeed, chlorpromazine has been shown to prevent the hypoglycemia and death in drum- and burn-shocked rats.[18]

Although the hypoglycemia could well account for the behavior and eventual death of the aggregated mice, the cause of the hypoglycemia remains to be explained. That the hypoglycemia is secondary to depleted liver glycogen stores is evidenced by the fact that liver glycogen depletion occurs before any signs of depression or hypoglycemia. Other studies[20] have shown that violent exercise, to the point of exhaustion, is accompanied by the depletion of liver and skeletal muscle glycogen stores. In the present study exaggerated motor activity does not account exclusively for the depleted tissue glycogen stores and hypoglycemia, since those animals that did not become depressed and continued to be very active did not develop hypoglycemia.

Selye[16] has implicated the catecholamines from the adrenosympathetic nervous system and the glucocorticoids from the adrenal cortex in the glycemic responses following various stressful procedures. It has been previously shown that the *d*-amphetamine-induced release of endogenous norepinephrine is enhanced in aggregated mice.[9] Furthermore, pretreatment with drugs that deplete tissues of their norepinephrine effectively prevents the hypoglycemia, liver glycogen depletion, and death of the aggregated mice. It would appear then that the massive release of catecholamines does play a role in these events. Although the role of the adrenal cortex in the biochemical events occurring in the tissues of aggregated mice has not been studied, it has been reported that adrenalectomy protects against the toxicity of *d*-amphetamine in aggregated mice.[14] Thus, both the sympathetic nervous system and the adrenal cortex may be involved.

If the hypoglycemia is an important factor in the death of the aggregated mice, then amphetamine might be expected to be more toxic in hypoglycemic animals. Conversely, maintenance of normal blood glucose levels in aggregated, *d*-amphetamine-treated mice might be expected to ameliorate the symptoms and to prevent death. Experiments to check these possibilities are currently in progress.

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