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Effects of stress and D-amphetamine on rat brain catecholamines*

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CERTAIN stressful environmental factors are known to influence the excitatory and lethal actions of D-amphetamine,[1, 2] and some of these influences may be exerted through the release of norepinephrine from endogenous stores.[3] For example, in individually caged mice, D-amphetamine causes partial depletion of brain and heart norepinephrine stores; aggregation or crowding of mice markedly enhances the toxicity and norepinephrine-depleting actions of this drug. We,[4] as well as others,[5] have reported that in 'nonstressed' rats D-amphetamine causes a reduction in the norepinephrine content of brain; it does not affect the brain content of dopamine. The present study was initiated in an effort to determine what effects various 'stresses' have on the ability of D-amphetamine to deplete the catecholamines in rat brain.

Sprague–Dawley female rats weighing 180–220 g were randomly divided into seven groups of twelve each (one control and six stressed groups). Each group was divided in half; six rats received an i.p. injection of D-amphetamine (3 mg/kg) in a volume of 5 ml isotonic saline/kg, and the other six received equal volumes of saline. The rats were then subjected to various stressful situations. After a period of 4 hr (1 hr for grid shock series) the animals were sacrificed and the norepinephrine and dopamine content of the brains determined as previously described.[4]

The stressful procedures used in this study were as follows.

Restraint. Rats were placed singly in adjustable wire-mesh cages that prevented changes in posture for a 4-hr period.

Swim. The animals were forced to swim in individual containers for 4 hr. The temperature of the water was maintained at 23° for one series (12 animals) and at 37° for another series (12 animals).

Tail shock. Four rats were placed in restraint cages and were wired in series with alligator clips attached to their tails. The tails were shocked every 5 sec with 175 V square-wave pulse of 1 sec duration (laboratory stimulator AEL 104A). Polarity was switched every 2 min. Under these conditions the shocks caused the rats to squeal and twitch their tails.

Sound. Rats were placed in individual cages in a small enclosed room and subjected to a loud tone (4,000 c/s) for 2 sec every 5 sec. The animals reacted to the tone for the first few minutes, but within 30 min adapted to it so that it no longer appeared to distress them.

Grid shock. Two rats (one treated with saline and one with D-amphetamine) were placed in separate compartments of a Skinner box containing a floor of stainless steel rods through which an electric shock could be delivered. Every 10 sec each rat was subjected to a shock of 1·6 mA intensity and 1 sec duration. Preliminary studies showed that the D-amphetamine-treated animals began to die if kept in the shocking cages for longer than 1 hr.

After exposure to the sound, restraint, and tail shock procedures, the general appearance of the saline- and D-amphetamine-treated rats was not different from the nonstressed rats receiving saline and D-amphetamine. There were no deaths in these series. In the swim series both the saline- and D-amphetamine-treated rats were depressed and exhausted at the time of sacrifice. Several animals

(both saline- and D-amphetamine-treated) drowned before the end of the 4 hr; they were not included in the results. However, they were replaced by additional animals in order to maintain the group size. There was a marked difference in the saline-treated and D-amphetamine-treated animals subjected to grid shock. Initially, the D-amphetamine-treated rats reacted more violently to the shocks (squealing and jumping). By the end of 1 hr they were quite depressed, often lying on the grid floor; the saline-treated rats were not depressed after 1 hr of shocking. A detailed study of the effects of grid shock on the toxicity of D-amphetamine has been reported by Weiss *et al.*[6]

The effects of the various procedures on the catecholamine content of rat brain are summarized in Fig. 1. The effects of D-amphetamine in restrained rats have been reported previously[4] but are included here for comparison. There was no statistical difference between the catecholamine levels in the brains of rats swimming at 23° and at 37°; the results of both series were combined for presentation in Fig. 1.

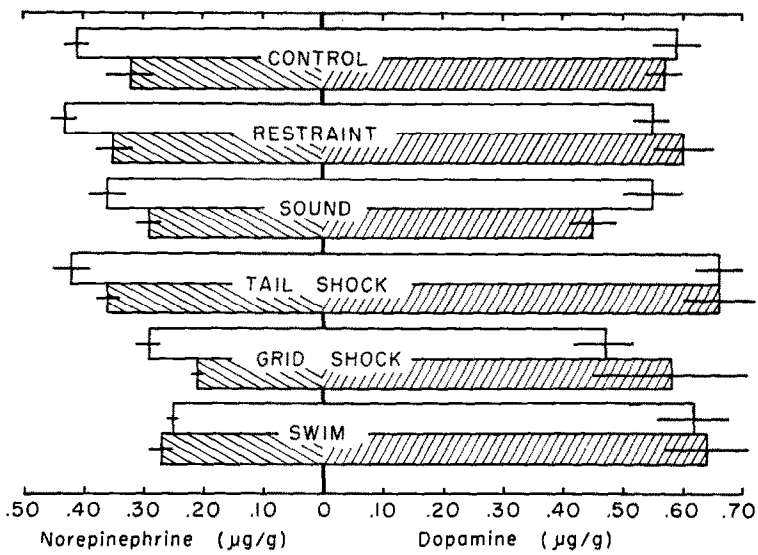

FIG. 1. Effect of D-amphetamine (3 mg/kg) on the catecholamine levels in the brains of rats subjected to various stressful situations. The length of each bar represents the mean; the horizontal line through each bar represents the standard error of the mean of catecholamines expressed as μg/g brain wt. The unshaded bars represent the saline-treated rats; the shaded bars the D-amphetamine-treated rats. Each bar represents the mean of 6 animals except in the swim series where it represents 12 animals.

In the saline-treated rats that were subjected to the various stressful situations, only those undergoing grid shock and the 4-hr swim had a significantly lower ($P < 0.01$) brain content of norepinephrine than did control nonstressed animals. These results confirm the previous findings of Maynert and Levy[7] and of Barchas and Freedman.[8] In the grid-shocked rats the brain norepinephrine level was reduced to a significantly greater extent ($P < 0.01$) after D-amphetamine pretreatment than after saline pretreatment. In all other series the norepinephrine depletion of the D-amphetamine-treated rats was not significantly different from similarly treated rats receiving saline. Although there was some variability in the brain levels of dopamine, no significant difference between groups could be demonstrated; this was true for both saline-treated and D-amphetamine-treated rats.

Although the mechanisms are not understood, it appears that both physical ('stress'-induced) and chemical (D-amphetamine-induced) stimuli[4] are capable of partially depleting brain stores of norepinephrine. The mechanisms by which both cause depletion may be the same. That is, certain drugs and stressful procedures might release norepinephrine through excessive stimulation, perhaps reflexly, of nervous pathways which transmit their impulses by means of norepinephrine. In this case, depletion

of norepinephrine occurs because it is released and destroyed at a faster rate than it is synthesized and stored. Dopamine, on the other hand, is not affected by these particular stimuli. This differential depletion of catecholamines in response to various stimuli can perhaps be explained by the fact that they are distributed differently within the brain. Norepinephrine is primarily located in the hypothalamus and other brain stem areas that are believed to represent the central component of the sympathetic nervous system; dopamine is primarily located in the basal ganglia—areas associated with the extrapyrimidal system. Drugs that deplete both catecholamines probably do so by interfering with the storage mechanisms or binding of the amines (e.g. reserpine). However, in the present study perhaps only neurons of the central sympathetic nervous system were stimulated and thus were partially depleted of their neurotransmitter—norepinephrine. Of course neither norepinephrine nor dopamine is a proven central transmitter substance so that this explanation of the depletion is only speculative.

Aggregation enhanced the toxicity of D-amphetamine in mice, This increased toxicity was accompanied by an increased depletion of brain and heart norepinepnrine stores.[3] Although a thorough examination of the toxicity of D-amphetamine was not made in the present study, it was apparent that grid-shocked animals were especially sensitive to the actions of D-amphetamine. Like the effects of aggregation in mice, grid shock in rats increased the toxicity and norepinephrine-releasing action of D-amphetamine, suggesting that under these conditions the excessive release of norepinephrine may be playing a role in the increased toxicity of D-amphetamine.

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

KENNETH E. MOORE
EUGENE W. LARIVIERE†

† Summer research fellow, supported in part by a grant from the New Hampshire Cancer Society. Present address: University of Rochester Medical School, Rochester, N.Y.

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Stimulation by acetylcholine and norepinephrine of glucose oxidation in rat submaxillary gland slices, as influenced by calcium

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THIS report directs attention to the importance of calcium in obtaining a stimulatory effect with neurohormones in the oxidation of ^{14}C-labeled glucose by rat submaxillary slices. Although Deutsch and Raper[1] had observed that acetylcholine increased the uptake of oxygen by cat submaxillary gland slices when glucose was available as a substrate, Sandhu[2] found that neither epinephrine nor acetylcholine increased the production of $^{14}CO_2$ in rat submaxillary slices incubated in Krebs-Ringer phosphate buffer (pH 7·4) from which the calcium was omitted. Since Douglas and Poisner[3] recently reported that calcium is required for the stimulation of secretion by acetylcholine and noradrenaline in perfused submaxillary glands of the cat, the study with rat submaxillary slices *in vitro* has been repeated, with particular attention to the presence of calcium in the medium.