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BRIEF COMMUNICATION

Effect of Metrazol on Brain Norepinephrine: A Possible Factor in Amnesia Produced by the Drug[1]

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PALFAI, T., P. KURTZ AND A. GUTMAN. *Effect of metrazol on brain norepinephrine: a possible factor in amnesia produced by the drug.* PHARMAC. BIOCHEM. BEHAV. 2(2) 261–262, 1974. — Metrazol-induced seizures reduced brain norepinephrine (NE) of mice trained in a passive avoidance paradigm. A reduction of NE was observed 5 but not 1440 min following injections. The implications of the results are discussed with respect to amnesia produced by metrazol.

Metrazol Norepinephrine Amnesia

SEIZURES induced by electroconvulsive shock (ECS) or pentylenetetrazol (metrazol) produce anterograde (AA) and retrograde (RA) amnesia. This means that retention is impaired when seizures are induced before or after a learning trial, with the degree of impairment dependent upon the temporal proximity of the training and the trauma [7, 8, 9, 10]. The specific mechanisms by which these seizures produce amnesia are not well understood. Although convulsions lead to several types of biochemical changes [1, 2, 3, 6] it is difficult to link these changes to the time-dependent effects of these agents on memory.

Recently Randt *et al.* [11] observed retention deficits when diethyldithiocarbamate (DDC) was given 30 min prior to passive avoidance training. The drug also decreased brain norepinephrine (NE) concentration along a similar time course as the appearance of the memory deficits. Therefore they suggested that a "noradrenergic compound" may be involved in memory formation of mice. Essentially the same conclusion was recently reached by others [4].

If this biogenic amine does play an important role in memory storage, one mechanism by which seizures could produce AA or RA is by altering brain NE levels during an early phase of memory formation. To investigate such a possibility, using metrazol, we determined the concentration of brain NE of mice trained in a passive avoidance paradigm, as a function of post seizure interval.

METHOD

Animals

Eighty-four adult male mice 70–100 days of age, obtained from Charles River Mouse Farms, Wilmington, Mass., were used. The mice spent at least 1 week in our animal colony before the experiment and were maintained under ad lib food and water conditions in a temperature (70–72°F) and humidity (50–70%) controlled room. A 12 hr day–night cycle was in effect.

Apparatus

The behavioral apparatus consisted of a covered V-shaped trough which was divided by a narrow guillotine door into a small illuminated start box and a larger darkened section. Stainless steel panels formed the walls and floor of the trough and served to deliver a 0.5 mA footshock (FS) for 2 sec in the darkened section. Fluorometric determinations were performed with an Aminco Model 4–7102 Fluoro-Microphotometer.

Procedure

Animals were individually placed into the start box for 1 min. Following this period, the door was opened. As soon as the animal stepped through into the darkened compart-

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ment, the door was closed and the mouse received either a FS or no footshock (NFS). Six groups (N = 6 in each) received FS, six groups were given NFS. Following this training, the animal was immediately removed from the apparatus and injected intraperitoneally (i.p.) with either 50 mg/kg metrazol or distilled water (DW). The animals were sacrificed 5, 60 or 1440 min following the injection and their brains were prepared for biochemical analysis. The experimental conditions make up the 2 × 2 × 3 factorial design (FS × Drug × Time) shown in Table 1. Two additional control groups were included in the experiment. Neither group received FS; one was given no injection, the other was treated with reserpine (2.5 mg/kg i.p.) 120 min prior to sacrifice.

All animals were sacrificed by decapitation. The brain was rapidly removed (within 150 sec) and plunged into 5 ml of 10% ice-cold trichloroacetic acid. The brain was then weighed, homogenized, centrifuged and following adjustments of reagent volumes, analyzed according to the laboratory manual provided by Bio-Rad Labs., Richmond, California. Bio-Rad Catecholamine Columns and reagents were used for the analysis. The NE concentrations were estimated fluorometrically using 405 and 490 mμ exciting and emitting filters respectively.

RESULTS AND DISCUSSION

Metrazol produced convulsions in approximately 90% of the animals injected. Animals which failed to convulse were replaced.

The results of the brain NE determinations are shown in Table 1. A 2 × 2 × 3 analysis of variance, using means as single observations [5], indicated a significant drug main effect ($F(1, 60) = 5.83$, $p<0.05$) but neither the FS nor the Time main effects were significant. The only other significant effect was the Drug × Time interaction ($F(2, 60) = 9.98$, $p<0.005$). Analysis of this effect indicated that metrazol reduced brain NE 5 but not 60 or 1440 min following the injections.

These data show that a convulsive dose of metrazol lowers brain NE in mice. Although the effect is temporary, it can occur in a period which may be critical for consolidation of memory.

In interpreting the results two points should be made. First, if NE is involved in memory formation in mice, as suggested by Randt *et al.* [11], one mechanism by which metrazol could produce amnesia is by altering brain NE during memory storage. Second, using the same paradigm, we have previously reported [10] AA and RA following metrazol injections. The time course of AA in that study was very similar to the time course of brain NE decrease and recovery following seizures in the present study. That is, AA could be produced if seizures occurred 60 min or less before training, but not if the interval was longer. While the similarity between the time courses in the two experiments may simply be a coincidence, the results are in agreement with the notion that if the normal level of brain NE is altered by seizures or drugs during an early phase of memory storage, retention impairment may result.

TABLE 1

THE EFFECT OF METRAZOL-INDUCED SEIZURES, DISTILLED WATER AND RESERPINE ON WHOLE BRAIN NE OF MICE

Condition	N	Brain NE μg/g	S.E. ±
NFS-Met-5	6	0.379	0.034
NFS-Met-60	6	0.412	0.048
NFS-Met-1440	6	0.474	0.068
NFS-DW-5	6	0.491	0.036
NFS-DW-60	6	0.455	0.069
NFS-DW-1440	6	0.476	0.038
FS-Met-5	6	0.386	0.074
FS-Met-60	6	0.464	0.058
FS-Met-1440	6	0.478	0.035
FS-DW-5	6	0.491	0.045
FS-DW-60	6	0.467	0.045
FS-DW-1440	6	0.414	0.087
No treatment	6	0.450	0.034
Reserpine 2.5 mg/kg	6	0.119	0.021

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