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Catecholamine Levels in the Whole Brain and the Probability of Memory Formation are not Related

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PALFAI, T., O. M. BROWN AND T. J. WALSH. *Catecholamine levels in the whole brain and the probability of memory formation are not related.* PHARMAC. BIOCHEM. BEHAV. 8(6) 717–721, 1978. – Amnesia was induced with reserpine or was blocked with l-dopa and 5-hydroxytryptophan before or after passive avoidance training in mice. The levels of dopamine and norepinephrine in the whole brain were measured in corresponding groups with gas chromatography-mass spectrometry. No correlation was found between retention and the levels of these catecholamines in the brain.

Amnesia Memory Reserpine Biogenic amines

THE catecholamines, dopamine (DA) and norepinephrine (NE), appear to be involved in memory formation [2, 14, 16]. Data supporting this notion come mainly from drug studies where a chemical with known effects on these neurotransmitters produces amnesia [1, 4, 5, 6, 8, 9, 10, 20]. It has been suggested that the levels of these catecholamines measured in the whole brain during or shortly after training predict retention performance [7, 21, 23].

Reserpine, among its many pharmacological effects, depletes DA and NE in the brain [12, 26, 27]. Since previous work has shown that the drug produces amnesia [17], we investigated the time and dose-related effects of reserpine on retention, blocked its amnesic effect with a combination of l-dopa and 5-hydroxytryptophan (5-HTP), and measured the levels of DA and NE following the same time intervals, drugs and dosages that produced these behavioral phenomena. Using a refined gas chromatography-mass spectrometry (GC-MS) procedure to measure DA and NE, we examined the relationship between the levels of these putative neurotransmitters in the whole brain and the probability of amnesia.

EXPERIMENT 1

The purpose of this experiment was to investigate the time and dose-related effects of reserpine on whole brain DA, NE and on retention.

METHOD

Animals

Adult (70–90 day-old) male albino mice bred from CD-1 stock in our animal colony were used. The animals were housed in groups of four in standard Econo plastic cages in temperature (70–72°F) and humidity (50–70%) controlled environment. Food and water were available ad lib and a 12 hr light/dark cycle was in effect.

Apparatus

A step-through passive avoidance apparatus, similar to that of Jarvik and Kopp [13] was used. Briefly, the apparatus consisted of a V-shaped trough that 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, in the darkened section, to deliver electric shock from a Grason-Stadler Model-700 Constant Current Shock Generator to the animals' feet.

Behavioral Procedure

For the behavioral portion of the experiment, 381 mice

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were injected intraperitoneally at 1 of 9 intervals before or after a single passive avoidance training with one of three doses (1, 2.5, or 4 mg/kg) of reserpine (Serpasil, Ciba). Two control groups were given training and injected with physiological saline either immediately or 1 hr following training. A third group was given pentylenetetrazol (50 mg/kg), a documented amnesic agent [18], immediately following training. A fourth control group was given saline 2 hr prior to being placed in the training apparatus, but no footshock was given.

Passive avoidance training consisted of placing the mouse into the illuminated start chamber; 60 sec later, the guillotine door was opened and the latency to step-through into the darkened section was recorded. Immediately following step-through (defined as the passage of the hind limbs over the threshold), the door was closed and the mouse given a 1 mA footshock for 3 sec. Retention was tested 7 days later, well after all the acute effects of the drug treatment had dissipated. Testing consisted of measuring step-through latency for an arbitrary maximum of 300 sec. This latency measure was used as the index of retention.

Chemical Procedure

Nine groups of mice (N = 113) were treated with one of three doses of reserpine (1, 2.5, 4 mg/kg) at 1 of 3 time intervals (1440, 120, 30 min) before the animals were sacrificed and their brains prepared for analysis. Two control groups received the drug vehicle at either 24 or 2 hr before sacrifice and one other group was sacrificed without treatment.

Catecholamines were extracted using a method similar to that of Schellenberger and Gordon [24]. Each mouse was decapitated, the brain rapidly removed and plunged into a preweighed homogenizing tube. The tube contained 5 ml $HC10_4$ homogenizing medium (1 g $Na_2S_2O_5$ + 0.5 g Na_2EDTA in 1 L 0.4N $HC10_4$) and 0.5 μg αMeDA (internal standard). The tube was weighed and the tissue was homogenized with a Willems Polytron PT-10 homogenizer (Brinkmann Instruments, Westbury, New York). The sample was centrifuged at 4°C for 20 min at 20,000 xg. The resulting supernatant was decanted into an extraction tube containing 300 mg conditioned alumina. (Prepared after Weil-Malherbe, [28]). A solution of 2.5 g Na_2EDTA in 100 ml of 0.525N NaOH (prepared fresh daily) was added until the pH was adjusted to 8.7. The mixture was shaken for 15 min and poured into 4 cm polypropylene column. The alumina with bound catecholamines was washed 3 times with 10 ml degassed glass-distilled water. The catecholamines were eluted from the alumina with 3 ml 0.2N acetic acid. The eluate was frozen and lyophilized.

PFPA derivatives of the catecholamines were made as reported by Koslow *et al.* [15]. Ethyl acetate (30 μl) and 100 μl PFPA (both redistilled and stored over Na_2SO_4) were added to the residue from a lyophilized sample. Sample vials were sealed and reacted for 1 hr at 60°C. The reaction mixture was blown dry with nitrogen and the derivatives were solubilized in 50 μl ethyl acetate.

A small aliquot (2–4 μl) of the solution was injected into a Finnigan 3100 Gas Chromatograph-Mass Spectrometer (GC-MS) equipped with a Model 6100 Interactive Control and Graphic Output plus a Multiple Ion Detector (Finnigan Corp., Sunnyvale, California). The GC column was a 5 ft x 4 mm ID silanized glass U-tube packed with 3% OV-17 on GCQ (100–120 mesh) (Applied Science Laboratories, Inc., State College, Pennsylvania).

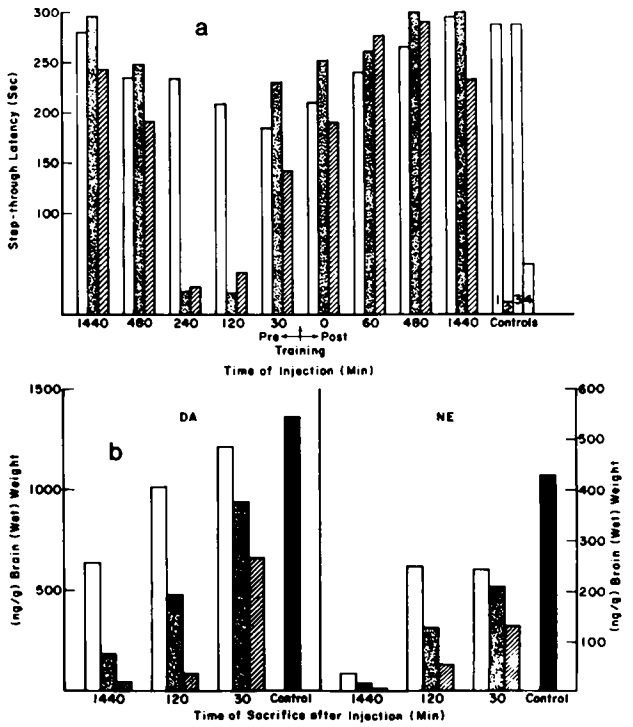

FIG. 1. (a) The time-dependent effect of three different doses of reserpine (Res) on retention of passive avoidance training are shown. (□ = 1 mg/kg; ⊡ = 2.5 mg/kg; ▨ = 4 mg/kg.) The 2.5 and 4 mg/kg doses given 4 or 2 hr prior to training produced amnesia 7 days later. None of the other reserpine groups were amnesic. The first and third control groups were given saline immediately or 1 hr following training. The second control group received 50 mg/kg pentylenetetrazol immediately following training; the 4th group was not given footshock but was treated with saline 2 hr prior to conditioning. (b) The effect of three doses of reserpine (Res) on the levels of dopamine and norepinephrine in the whole brain are shown as a function of time. (□ = 1 mg/kg; ⊡ = 2.5 mg/kg; ▨ = 4 mg/kg.) The results of the GC-MS quantification of catecholamines were expressed in ng/g brain tissue. Despite the severe depletion of both catecholamines resulting from the 4 mg/kg dose 24 hr following injection, the comparable groups in the behavioral study showed good acquisition and retention of the training. (Dopamine: Dose effect = $F(2,79) = 51.82$, $p<0.001$; Time effect = $F(2,79) = 53.61$, $p<0.001$; Interaction = not significant). (Norepinephrine: Dose effect = $F(2,38) = 5.95$; $p<0.005$; Time effect = $F(2,38) = 15.02$; $p<0.001$; Interaction = not significant).

GC-MS conditions were as follows: column temperature 200°C; injector port temperature 240°C; separator oven temperature 220°C; transfer line temperature 220°C; electron energy 70 eV; ion energy 7.5 V; emission current 0.60 mA; and helium flow rate 15 ml/min. Standard curves for NE and DA quantitation were developed by adding increasing amounts of NE and DA to tubes containing 0.5 μg αMeDA internal standard, 0.5 ml 0.9% saline, and 5 ml $HC10_4$ homogenizing medium. The standard solutions were carried through the method as described above. Standard curves for NE were constructed by plotting the ratio of the height of the m/e 176 NE (mass fragmentogram) peak to the m/e 190 αMeDA peak against μgs of NE (free base) added. Similar curves were constructed for DA by plotting

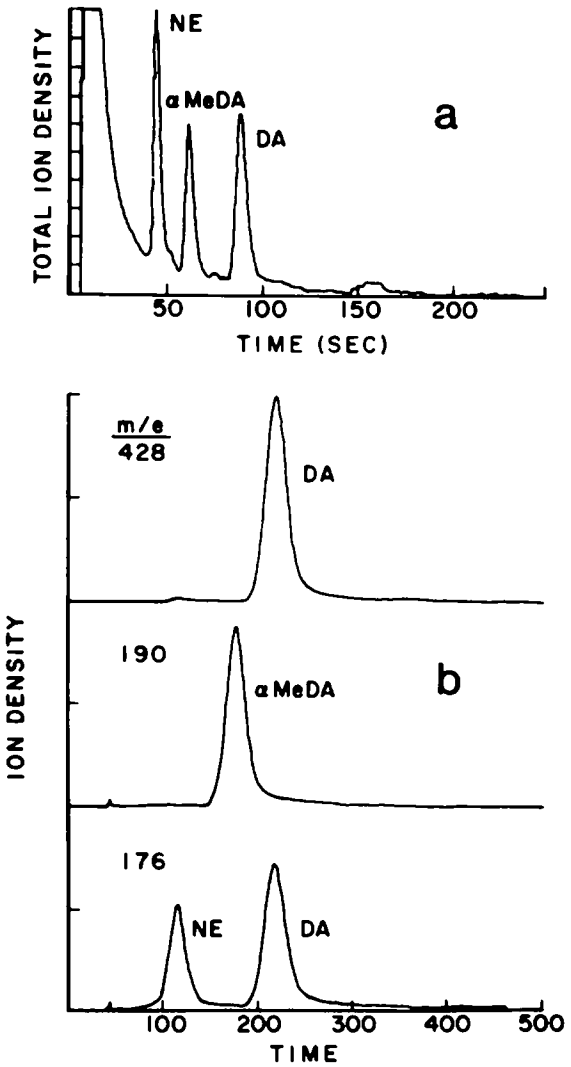

FIG. 2. (a) Gas Chromatogram of PFPA Derivatives of NE, αMeDA, and DA are shown. PFPA derivatives were prepared for GC-MS as described, and monitored with the total ion monitor. (b) Mass Fragmentogram of PFPA derivatives of NE, αMeDA, and DA are shown. The ions selected for mass fragmentography monitoring were: NE, m/e 176; αMeDA, m/e 190; and DA, m/e 176 and m/e 428.

the ratio of the height of the m/e 428 DA peak to the height of the m/e 190 αMeDA peak against μgs of DA (free base) added.

RESULTS

Reserpine impaired retention significantly only when given in doses of 2.5 or 4 mg/kg, 4 or 2 hr prior to training (Fig. 1a). A Kruskal-Wallis nonparametric analysis of variance and a group-by-group comparison via a series of Mann-Whitney U-tests supported this conclusion. We have shown elsewhere that this amnesic effect could not be attributed to either state-dependent learning, altered footshock sensitivity or a possible debilitating effect of the drug [19]. We suggested at that time that the time-dependent central depletion of biogenic amines could be responsible for the amnesic gradient.

The GC-MS method reported here [15] resulted in good chromatographic separation of the pentafluoropropionic anhydride (PFPA) derivatives of NE, αMeDA and DA (Fig. 2a). The mass spectra of the compounds responsible for these chromatographic peaks are similar to those reported to describe the chemical structures of PFP-NE, PFP-αMeDA and PFP-DA [15]. The identification of NE and DA isolated from mouse brain was done by comparing the mass spectra of PFPA derivatives from brain extracts with those of authentic PFP-NE and PFP-DA. By using an internal standard (αMeDA) and multiple ion detector on the GC-MS, we were able to measure NE and DA by the method of quantitative mass fragmentography [11]. A sample mass fragmentogram is given in Fig. 2b.

The concentrations of DA and NE in the brain of the epxerimental and control groups are given in Fig. 1b. Maximum depletion occurred when the highest dose (4 mg/kg) was given 24 hr prior to sacrifice. At this interval the same dose, however, did not produce amnesia (Fig. 1a). Furthermore, the levels of DA and NE obtained after 24 hr following the 1 mg/kg dose (that never produced amnesia) were comparable or even lower than those following the 2.5 mg/kg dose given 120 min before sacrifice. The latter dose and time interval produced retention impairments in the behavioral experiment. These results suggest that the amount of DA or NE in the brain during conditioning is not related to the probability of memory formation of passive avoidance training.

EXPERIMENT 2

Previously we reported that the reserpine-induced amnesia could be blocked with a combination of l-dopa and 5-HTP given in close temporal proximity even following the training trial [19]. L-dopa is the metabolic precursor of DA and NE; 5-HTP is for serotonin (SE). It was suggested [1, 4, 5, 11] that the precursors might have prevented the depletion of these putative neurotransmitters and thus were able to block the amnesic effect of reserpine. We replicated the behavioral experiment and simultaneously measured the effect of l-dopa and 5-HTP on the levels of catecholamines in the brain of both normal and reserpine-treated animals.

METHOD

Animals

Mice of the same description as previously, were used.

Apparatus

Both the behavioral and chemical apparatus were the same as before.

Procedure

Four groups of mice (N = 53) were given 2.5 mg/kg reserpine 120 min before passive avoidance training. They also received a second drug treatment in that 3 groups were given 100 mg/kg l-dopa and 125 mg/kg, d, l, 5-HTP either 15 min before or 10 or 90 min after passive avoidance training. Retention was measured 7 days later.

In the biochemical counterpart of this experiment, 30 mice were treated with either 2.5 mg/kg reserpine or the drug vehicle, and 105 min later, the precursor combination, l-dopa and 5-HTP, was given. The animals were then sacrificed at either 15, 30 or 90 min following the precursor treatments.

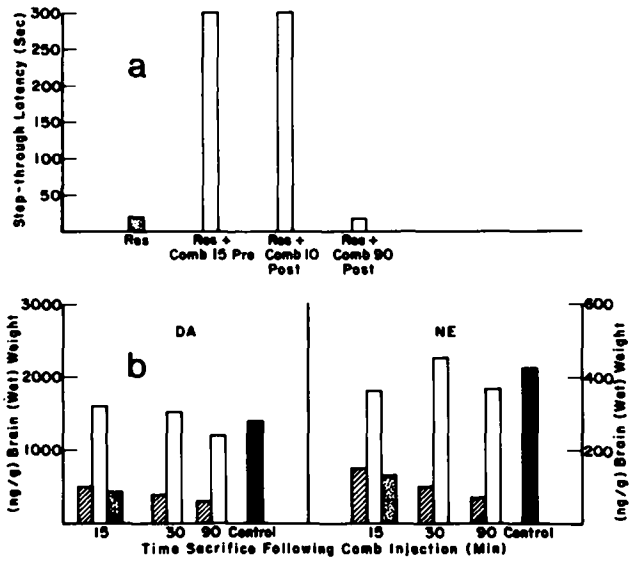

FIG. 3. (a) The effect of reserpine (2.5 mg/kg) alone (Res) and with a combination of neurotransmitter precursors, l-dopa (100 mg/kg) and 5-HTP (125 mg/kg) on retention is shown. The precursor combination given 15 min before (comb 15 pre) or 10 min after (comb 10 post) training was able to block the reserpine-induced amnesia. When given 90 min following training (comb 90 post), the amnesia effect was not blocked. (b) The effect of the precursor combination (comb) on the levels of DA and NE of the whole brain in reserpine (▨ = reserpine + combination) or saline-treated (□ = saline + combination) animals is shown. The precursor combination could neither reverse the effect of reserpine on the levels of catecholamines nor significantly elevate them in vehicle-treated controls. For comparative purposes, the 2.5 mg/kg dose of reserpine given 2 hr prior to sacrifice without precursors from Fig. 1 b, is included in the Figure. (Dopamine: Treatment effect = $F(1,22) = 262.0$; $p<0.001$; Time effect = $F(2,22) = 5.45$; $p<0.01$; Interaction: not significant). (Norepinephrine: Treatment effect = $F(1,22) = 37,48$; $p<0.001$; Time effect and Interaction: not significant).

In agreement with our previous findings [19], the reserpine-induced amnesia could be blocked with the administration of l-dopa and 5-HTP when these precursors were given 15 min before or 10 min after passive avoidance training but not when given 90 min later (Fig. 3a). The effect of the precursor combination in the 10 min posttraining group is of special significance, first because it suggests that reserpine-treated animals were capable of learning (i.e. they fully experienced the training) and, second, because it implies that in this instance reserpine interferes with memory formation at some phase later than 10 min following the training.

The measured catecholamine levels following the drug treatments are shown in Fig. 3b. It is apparent that the precursor combination was able to block amnesia but it had no effect on the brain levels of DA and NE in reserpine-treated animals. This implies that l-dopa and 5-HTP attenuate the reserpine-induced amnesia by pharmacological mechanism other than the elevation of depleted catecholamines. While this still leaves the possibility that the blockade of the amnesic effect was mediated through serotonin, elevation of this putative transmitter in the brain should lead to impairment rather than improvement of retention [3,22].

DISCUSSION

The results of these experiments indicate a time and dose dependent effect of reserpine on retention and the levels of whole brain catecholamines. However, no relationship appears to exist between the behavioral and biochemical effects of the drug. The implication of this finding is that the levels of whole brain catecholamines during training do not predict subsequent retention performance in the mouse.

The conclusion is indirectly supported by the outcome of the second experiment. The combination of the biogenic amine precursors, l-dopa and 5-HTP, was able to attenuate the reserpine-induced amnesia. However, at the dose levels used here, these precursors did not affect the levels of whole brain catecholamines either in saline or in reserpine-treated animals. The observation suggests again that the probability of retention performance is not related to the levels of catecholamines in the brain of the mouse.

The various pharmacological effects of a myriad of amnesic agents offer a wide choice among hypothetical mechanisms to explain amnesia. Many of these agents affect the level, synthesis, turnover, reuptake or metabolism of DA and NE, and perhaps for this reason, memory mechanisms mediated by these catecholamines seem attractive as a working hypothesis. If catecholamines are involved in memory, then their functional availability in the brain rather than their overall levels would be important.

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