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Effect of an Amnesic Dose of Reserpine, Syrosingopine or Guanethidine on the Levels of Whole Brain Dopamine and Norepinephrine in the Mouse

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BROWN, O. M., T. PALFAI AND L. WICHLINSKI. *Effect of an amnesic dose of reserpine, syrosingopine or guanethidine on the levels of whole brain dopamine and norepinephrine in the mouse.* PHARMAC. BIOCHEM. BEHAV. 15(6) 911–914, 1981.—Independent groups of mice were treated with an amnesic dose of either reserpine, syrosingopine or guanethidine. The animals were sacrificed either 2 or 24 hrs later. While reserpine depleted the levels of whole brain dopamine and norepinephrine at both times, syrosingopine or guanethidine did not appreciably deplete these catecholamines at either time. Since all three drugs produce amnesia when given 2 hrs before passive avoidance training, it appears that the levels of whole brain catecholamines at the time of training do not predict the probability of memory formation.

Biogenic amines Norepinephrine Dopamine Rauwolfia alkaloids Guanethidine Amnesia

LIKE others [1, 3, 6, 20], we reported that reserpine, syrosingopine or guanethidine produced time-dependent retention impairments in mice [9, 15–18, 22–24]. Since these compounds possess considerable antiadrenergic properties, several investigators suggested that it is this action of the above drugs that produces the observed retention deficits [1, 3, 5]. We qualified this idea by proposing that the antiadrenergic effects need not occur in the brain; the peripheral actions of reserpine, syrosingopine or guanethidine might be sufficient to account for their amnesic effects.

Support for this peripheral hypothesis comes from the following observations. (1) We reported that a reserpine injection (2.5 mg/kg) which depleted 90–95% of the brain catecholamines 24 hrs following its administration, did not result in amnesia. When the same dose was given 2 hrs before sacrifice or behavioral training, amnesia was observed during subsequent testing 1 week later, although the depletion of dopamine (DA) and norepinephrine (NE) was only 60%. Apparently the levels of whole brain catecholamines at the time of memory formation did not predict the probability of retention [16]. (2) We also reported that when DA or NE were administered peripherally either before or even shortly after training, the amnesic effects of reserpine, syrosingopine or guanethidine [17, 23, 24] could be blocked, i.e., retention of the training was not impaired. Since the peripherally-administered catecholamines presumably do not cross the blood-brain barrier [25], it seems that increasing the levels of peripheral catecholamines during or shortly after training is sufficient to attenuate the amnesic effects of these antiadrenergic compounds. (3) Finally, several publications indicate that the actions of syrosingopine or guanethidine are restricted to the periphery, since their administration reportedly does not alter brain catecholamine levels [4, 8, 10–13]. However, we cannot be certain that under our experimental conditions these peripheral drugs do not affect the brain levels of DA or NE in mice.

The purpose of the present study was, therefore, to investigate the effects of reserpine, syrosingopine and guanethidine on the levels of DA and NE in the brain of the mouse by utilizing dosages and time intervals with previously demonstrated behavioral effects.

METHOD

Subjects

The experiment was performed on 70–100 days old male White Swiss mice bred in the Psychology Research Laboratory at Syracuse University from parent stock of the CD-1 strain originally obtained from Charles River Breeders, Wilmington, MA. The mice were housed in standard Econo-plastic cages, four to six per cage, in a temperature (21°C) and humidity (50%) controlled environment. Purina laboratory chow and tap water were continuously available and a 12 hr light-dark cycle was in effect (6 a.m.–6 p.m. on).

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Pharmacological Procedure

A total of 81 animals was used. Animals were injected intraperitoneally with either 2.5 mg/kg reserpine (Serpasil, CIBA), 2.5 mg/kg syrosingopine (Singoserp, CIBA), 40 mg/kg guanethidine (Ismelin, CIBA), or 10 ml/kg body weight of drug vehicle. The vehicle was 200 mg ascorbic acid and 100 μl Tween 80 per 20 ml sterile water. All drugs were prepared fresh daily. Injections were made either 2 or 24 hrs before sacrifice. The animals were sacrificed by spinal dislocation between 12 p.m. and 2 p.m.

Chemical Procedure

Catecholamines were extracted using an abbreviated version of our previous method [16] which does not involve an alumina binding procedure [2]. Following decapitation, the brain was rapidly removed from each mouse and plunged into a preweighed homogenizing tube. The tube contained 6 ml $HClO_4$ homogenizing medium (0.55 g $NaHSO_3$ in 1 L 0.4 N $HClO_4$, prepared fresh daily) and 160 ng (as free base) 3,4-dihydroxybenzylamine (DHBA) as an internal standard. The tube was reweighed and the tissue was homogenized with a Willems Polytron PT-10 homogenizer (Brinkman Instruments, Westbury, NY). The sample was centrifuged at 4°C for 20 min at 20,000 × G and the resulting supernatant was decanted into a clean tube. An aliquot of this supernatant was analyzed for catecholamine content by High Performance Liquid Chromatography (HPLC).

High Performance Liquid Chromatography (HPLC)

The catecholamines from our samples were separated by HPLC and detected with an electrochemical (EC) device. The HPLC apparatus has the following components in series: reservoir for mobile phase; pump for mobile phase (Simplex Mini Pump, Milton Roy Co., Riviera Beach, FL); 100 feet of 0.8 mm i.d. teflon tubing (for pulse dampening); a 20 μl slide valve for sample injection (Laboratory Data Control, Englewood Cliffs, NJ); a precolumn [14]; a glass column (500 mm long × 2 mm i.d.) filled with Vydac SC cation exchange packing (The Separations Group, Hesperia, CA); and a modified electrochemical thin layer transducer (EC) with a carbon paste electrode (Bioanalytical Systems, West Lafayette, IN). The EC was operated with a Bioanalytical Systems LC-2A Amperometric Controller, the output of which is displayed on a strip chart recorder. The apparatus except for the reservoir, pump, controller and recorder is contained within a Faraday Cage.

The mobile phase was an acetate-citrate buffer at pH 5.1 [21] flowing at 0.3 ml/min. The EC was operated in the oxidation mode at +0.5 V.

Standard curves for NE and DA quantitation were developed by adding increasing amounts of NE and DA to tubes containing 160 ng DHBA internal standard, 0.5 ml 0.9% saline, and 6 ml $HClO_4$ homogenizing medium. (NE, DA and DHBA were obtained from Sigma Chemical Co., St. Louis, MO.) 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 NE HPLC peak to the DHBA peak against ng of NE (as free base) added. Similar curves were plotted for DA by plotting the DA/DHBA peak height ratio against ng of DA (as free base) added.

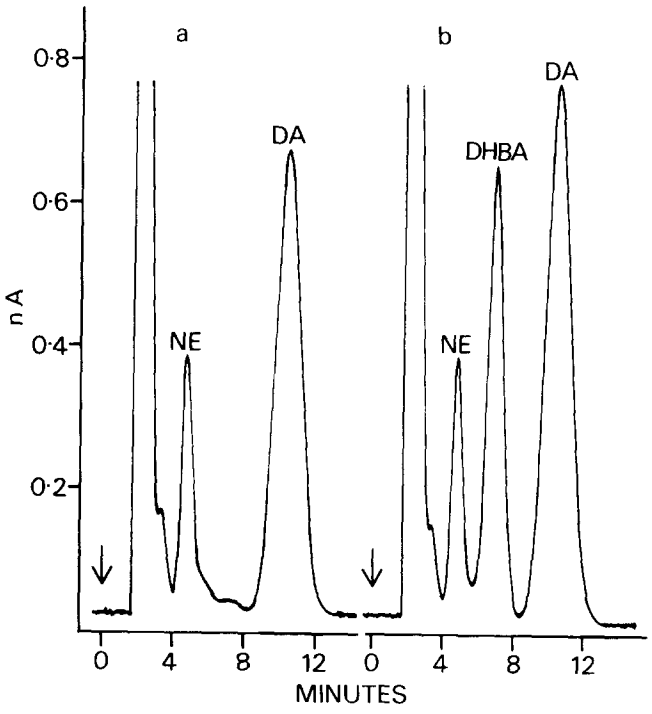

FIG. 1. HPLC-EC chromatograms of (a) mouse brain extract, and (b) mouse brain extract with 320 ng DHBA added as internal standard. The moment of injection is indicated by the arrow (↓) and the ordinate represents EC dectector current output in nanoamps (nA). Conditions for chromatography are given in the Method section.

RESULTS

The rapid HPLC method reported here avoids the lengthy alumina binding procedure and results in clean, symmetrical chromatographic peaks for catecholamines extracted from mouse brain. Figure 1 indicates good separation between HPLC peaks for brain NE and DA and the internal standard, DHBA. This rapid HPLC method yields the same values for NE and DA analysis as those obtained with alumina binding and gas chromatography-mass-spectrometry [16].

With this no-alumina method, a substance is extracted from mouse brain which has the same HPLC retention time as NE. Voltammogram studies indicated that this compound has an oxidation current plateau at approximately +0.8 V while the plateau for NE and DA is about +0.5 V. Thus, by operating our EC at +0.5 V we were able to quantitate NE with no interference from the unknown substance. This substance was not depleted from brains by reserpine or syrosingopine. The unknown does not bind to alumina, and it has an HPLC retention time different from normetanephrine, 4-hydroxy-3-methoxyphenylglycol, and octopamine.

The results of the catecholamine analyses are shown in Table 1. These data indicate that reserpine causes a dramatic depletion of brain NE and DA at both 2 and 24 hrs after injection. This depletion is not apparent in syrosingopine- or guanethidine-treated mice.

The NE and DA data were analyzed using separate 2×4 ANOV's, and the Greenhouse-Geisser Conservative F-Test was employed [26]. An overall effect was found for NE,

TABLE 1

WHOLE BRAIN CATECHOLAMINE LEVELS IN MICE AFTER DRUG TREATMENT
ng/g ± SD

Time	Control		Reserpine		Syrosingopine		Guanethidine	
	NE	DA	NE	DA	NE	DA	NE	DA
2 hrs	432 ±43	1230 ±187	97 ±41	274 ±104	373 ±55	1220 ±220	419 ±46	1360 ±240
	(n=18		(n=9		(n=9)		(n=9)	
24 hrs	384 ±53	1340 ±230	31 ±10	132 ±65	381 ±65	1350 ±120	361 ±35	1480 ±150
	n=11)		(n=8)		(n=9)		(n=8)	

$F(1,7)=102.51$, $p<0.0001$. The effect due to drug treatment was significant, $F(1,7)=228.94$, $p<0.0001$. A time-dependent effect in NE levels was also significant, $F(1,7)=15.57$, $p<0.01$, but no drug × time interaction was observed, $F(1,7)=2.26$, $p>0.10$.

A significant main effect was also seen for DA, $F(1,7)=75.72$, $p<0.0001$. The effect due to drug treatment was significant, $F(1,7)=173.83$, $p<0.0001$, but no significant time-dependent effect, $F(1,7)=2.69$, $p>0.10$, or drug × time interaction, $F(1,7)=2.43$, $p>0.10$, was present.

Since no drug × time interactions were found for either NE or DA, the data for treatment groups were pooled across time, and differences between control and treatment groups were analyzed using Dunnett's procedure for unequal n's [7] with α set at 0.01. Significant differences were found between vehicle- and reserpine-treated animals for both NE and DA. No significant differences were seen between vehicle- and syrosingopine-treated animals for either catecholamine, nor were any significant differences found between vehicle- and guanethidine-treated mice.

DISCUSSION

We have found that while reserpine depletes catecholamines, neither syrosingopine nor guanethidine produces a significant decrease in the whole brain levels of DA or NE in the mouse. The results of the controls and reserpine groups obtained here are the same as in our previous report, where we used a conventional alumina binding procedure with a gas chromatograph-mass spectrometer technique to measure catecholamines in the mouse brain [16]. Therefore, in addition to avoiding a lengthy alumina binding procedure, the HPLC technique described here is also very reliable.

As we reported previously [16] and have once again shown here, brain NE and DA are severely depleted at 2 and 24 hrs after an injection of reserpine. However, reserpine produces amnesia only when given 2 hrs, but not when given 24 hrs before training. We therefore suggested that the levels of whole brain catecholamines in the mouse do not predict the probability of retention.

Syrosingopine or guanethidine, in the doses given here, produces significant retention impairments when administered 2 hrs before a passive avoidance training trial [18,23]. However, the present experiment demonstrates that the levels of brain catecholamines did not differ from the controls following injection of either of these two drugs. Together with the behavioral results, these data imply that the amnesic effects of these drugs are not mediated by central catecholamines.

The question is then, by what mechanisms do these drugs produce retention deficits for a passive avoidance training in the mouse? Since we have shown that the amnesic effects produced by these drugs are not due to state-dependent learning, altered footshock sensitivity or chronic toxicity at the time of testing [9,15], the following explanations may be offered. First, the amnesic effects of these antiadrenergics are mediated centrally by mechanisms other than their effects on catecholamines. Second, changes in catecholamine turnover or localized changes in catecholamine levels (not detected with whole brain studies) may be involved in these effects. Third, it is possible that the amnesic effects are mediated through the peripheral antiadrenergic effects of these drugs. They may prevent a normal peripheral sympathetic reaction to an aversive conditioning trial, an effect that could have significant central consequences. This implies that peripheral catecholamines have a critical modulating influence on memory formation for aversively motivated tasks. Since we are able to reverse the amnesic effects of these drugs by administering catecholamines peripherally as long as 10 min following training [17,23,24], the peripheral sympathetic involvement in a training trial appears to play a critical time-dependent role in memory formation.

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