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BRIEF COMMUNICATION

Effects of Amnesic Doses of Reserpine or Syrosingopine on Mouse Brain Acetylcholine Levels

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PALFAI, T., L. WICHLINSKI, H. A. BROWN AND O. M. BROWN. *Effects of amnesic doses of reserpine or syrosingopine on mouse brain acetylcholine levels.* PHARMACOL BIOCHEM BEHAV **24**(5) 1457–1459, 1986.—The effects of reserpine and syrosingopine on mouse whole brain acetylcholine levels were examined. At 2 or 24 hr following injection, the brains were removed and analyzed by mass spectrometry. No differences were found between drug-treated and control mice in the acetylcholine content of the brain at either time interval. The results suggest that whole brain acetylcholine levels do not predict the amnesic effects of either reserpine or syrosingopine.

Acetylcholine Mouse brain Amnesia Reserpine Rauwolfia alkaloids

STUDIES from other laboratories [11], as well as from ours [16,18], have demonstrated reserpine to be an amnestic agent. The mechanism by which reserpine produces amnesia is unknown. Since reserpine depletes central biogenic amines [4], this effect could be responsible for its amnestic properties. However, we reported that reserpine administered 2 hr prior to passive avoidance training produced amnesia in the mouse, but failed to do so when administered 24 hr prior to training [20]. Since central catecholamine depletion was greater at 24 hr following injection than at 2 hr, the catecholamine levels were clearly not predictive of amnesia [15]. Furthermore, amnesia induced with reserpine could be reversed by a combined peripheral IP administration of the biogenic amine precursors, L-dopa and 5-hydroxytryptophan [18], in doses that had no effect on central catecholamine levels following depletion by reserpine [15]. These findings led to the suggestion that the peripheral antiaminergic properties of reserpine might account for its amnestic properties in passive avoidance situations [18].

Support for this hypothesis came from more recent findings in that systemic administration of norepinephrine or dopamine were shown to attenuate reserpine-induced amnesia for passive avoidance training in the mouse [17], even though these amines do not readily cross the blood-brain barrier [14]. Furthermore, syrosingopine, an analog of reserpine with predominantly peripheral action [4,16], produced amnesia in this task and its effect was also reversed by systemic norepinephrine or dopamine administration [21]. Finally, the doses of norepinephrine or dopamine sufficient to attenuate reserpine-induced amnesia were shown not to alter brain catecholamine levels significantly following reserpine pre-treatment [18]. These results have led us to suggest that, in a passive avoidance situation, peripheral catecholamines play a critical role in reserpine- or syrosingopine-induced amnesia.

Some of the amnesic effects of reserpine, however, must be mediated centrally. In discriminated escape-reversal task, where the animals were required to recall their previous choices and cognitive memory was called for, peripherally administered catecholamines did not reverse the amnesic effects of reserpine [20]. Central catecholamines may not play a role in memory formation, since their levels do not predict the probability of memory formation [15]. It is possible that mechanisms by which reserpine produces amnesia involve neurotransmitters other than catecholamines. Serotonin is a candidate since p-chlorophenylalanine, which was reported to have depleted the brain levels of this neurotransmitter [6], facilitated memory formation [19]. Acetylcholine (ACh) could be another, since it is frequently implicated in mechanisms of learning and memory [5,9]. That reserpine might

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TABLE 1

EFFECTS OF RESERPINE OR SYROSINGOPINE ON WHOLE BRAIN ACETYLCHOLINE LEVELS IN THE MOUSE (nmol/g, MEAN ± SEM)

	Treatment		
Time	Control	Reserpine	Syrosingopine
2 hr	19.7 ± 1.0 (n=11)	22.9 ± 1.2 (n=11)	18.9 ± 1.2 (n=11)
24 hr	20.6 ± 1.1 (n=11)	21.3 ± 1.0 (n=12)	21.2 ± 1.4 (n=11)

No significant differences using 2-way ANOVA.

exert some of it amnesic effects via cholinergic mechanisms is supported by the fact that elevated ACh levels were found in the hypothalamus, temporal lobe and cerebellum, but not in the hippocampus of dogs 3 hr after reserpine administration [11]. Elevated ACh levels have also been found after reserpine administration in cortical areas of dogs [10], rat brain stem [12] and rat brain (minus cerebellum, pituitary and olfactory lobe) [8]. However, no increases in ACh content of guinea pig cortex, caudate, thalamus, hypothalamus or brain stem were found at 2, 4 or 24 hr after IP injection of reserpine [1]. In fact, decreases were observed in the cortex at 4 hr and in the caudate at 4 and 24 hr after injection. Twenty-four hours after IP administration of reserpine to the rat, reductions of 13–18% in ACh levels were also found in several brain regions including striatum, midbrain, hypothalamus, hippocampus and pons-medulla [7].

Apparently, reserpine is capable of producing changes in brain ACh levels across a variety of species. From the studies cited here it is clear that there is no censensus on the direction of these changes. We investigated the effects of reserpine on mouse brain ACh levels in conditions where reserpine was shown to produce amnesia. The effects of syrosingopine on central ACh were also examined, since this drug did not produce amnesia in the discriminated escape reversal paradigm, but did in passive avoidance.

METHOD

Male albino mice (90–103 days, 30–45 grams) from the colony at the Behavioral Neuroscience Lab at Syracuse University were used in all experiments. All mice were kept on a 12:12 hr light:dark cycle and provided with food and water ad lib. Animals were injected (2.5 mg/kg, IP) with either reserpine (SERPASIL, CIBA), syrosingopine (SINGOSERP, CIBA) or an equal volume of vehicle 2 or 24 hr before sacrifice.

Reserpine was dissolved in distilled water, while syrosingopine was solubilized with 1% ascorbic acid and 2 drops of Tween 80 in distilled water. As a control for the assay method, another group of mice from the colony was injected with either pentobarbital (n=6), which has a known elevating effect on ACh levels [13], or saline (n=6) and analyzed in precisely the same fashion as the experimental group.

The mice were killed by cervical dislocation and then decapitated. Brains were rapidly removed and plunged into tubes containing 6 ml of acetonitrile, 120 nmol of tetramethylammonium iodide, 0.5 ml of 10^{-4} N HCl and 10 nmol of propionylcholine iodide (Sigma, St. Louis, MO) as internal standard. To generate a standard curve, increasing amounts (2–20 nmol) of acetylcholine iodide (Sigma) were added to tubes with the same contents.

Mouse brains were homogenized with a Willems PT-10 homogenizer (Brinkmann Instr., Westbury, NY) and centrifuged for 20 min at 20,000 × g (0–4°C). Brain samples and standards were extracted and prepared for ACh analysis by using procedures similar to those described previously [2,3]. The ACh and propionylcholine in each sample were precipitated, washed with ether, solubilized in acetonitrile and pipetted onto a platinum pyrolysis ribbon for analysis.

All samples were analyzed for ACh and propionylcholine content by pyrolysis mass fragmentography. A Finnigan 3100 gas chromatograph/mass spectrometer (Finnigan Corp., Sunnyvale, CA) fitted with a pyrolyzer, data system and a multiple ion detector was used in these studies. The operating conditions of the instrument have been specified in detail earlier [2,3].

The pyrolysis mass fragmentogram peak area ratios (acetylcholine/propionylcholine) were calculated for each sample and compared to the standard curve. Levels of ACh were determined and are expressed as nmol ACh/g (mean±SEM).

RESULTS

The results of the reserpine and syrosingopine treatment on mouse brain ACh levels are shown in Table 1. These data indicate that neither of the rauwolfia alkaloids had an effect on whole brain ACh levels at 2 or 24 hr post-injection. A 2×3 analysis of variance testing of these data indicated no significant main effect, $F(5,61)=1.48, p>0.05$; therefore, no further statistical analysis was performed.

The group of animals injected with pentobarbital had higher brain levels of ACh (47.9±4.6 nmol/g, mean±SEM) than did the matched control group (18.6±3.8 nmol/g) ($p<0.001$, t-test).

DISCUSSION

These results show that reserpine or syrosingopine, at doses and times which have previously been shown to produce amnesia, did not alter whole brain levels of acetylcholine. It should be noted that the analytical methods used here were sensitive to drug-induced changes in whole brain ACh levels. Pentobarbital administration produced a dramatic increase in whole brain ACh content as has been reported by others [13]. Therefore, failure to find changes in brain ACh resulting from reserpine or syrosingopine administration cannot be attributed to insensitivity of the method.

The hypothesis that some of the amnestic effects of these rauwolfia alkaloids might be mediated by a cholinergic mechanism was not supported. These findings along with those previously reported from our laboratory imply that whole brain levels of either norepinephrine, dopamine, or ACh do not predict the probability of memory formation after treatment with amnesic doses of reserpine [15]. It is possible that other neurotransmitter systems are involved in the mechanism of reserpine-induced amnesia. It is also possible that striking neurotransmitter changes may occur in discrete areas of the brain and may be masked in determinations of whole brain levels. Thus, measurements of whole brain levels of neurotransmitters may not be reliable predictors of memory processes in the brain.

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