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Short communication

DIFFERENTIAL EFFECTS OF d-AMPHETAMINE ON BRAIN ACETYLCHOLINE IN YOUNG, ADULT AND GERIATRIC RATS

Michael R. VASKO, Laurence E. DOMINO and Edward F. DOMINO
Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

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Varying doses of d-amphetamine were given i.p. to young (28 day old), adult (approximately 90 day old), and geriatric rats (approximately 2 years old). 30 min after injection, rats were sacrificed and ACH extracted and assayed by pyrolysis gas–liquid chromatography. Total brain acetylcholine (ACh) levels were not significantly different among the control animals in all three groups. d-Amphetamine, however, decreased total levels of ACh in young and geriatric rats without changing levels in adult animals.The changes in young rats were dose-related. These results suggest an important difference in brain cholinergic mechanisms depending upon age.

d-Amphetamine Acetylcholine Pyrolysis–gas chromatography Geriatric

1. Introduction

Recently, Consolo et al. (1972) reported that d-amphetamine in doses of 7.0 and 15.0 mg/kg i.p. had no effect on steady state brain levels of acetylcholine (ACh) in male albino mice. These data, although in another species, conflict with results of Domino and Wilson (1972) and Domino and Olds (1972), who were able to show significant decreases in the total levels of brain ACh in young and old rats, respectively.

Aside from species differences, the methods of sacrifice and assay were also different. Consolo's group sacrificed by freeze immersion and assayed by the enzymatic method of Saelens et al. (1970), while Domino's group sacrified by decapitation and used a modified frog rectus bioassay (Dren and Domino, 1968).

This manuscript attempts to establish the effects of d-amphetamine on rats as a function of their age, using a pyrolysis gas–liquid chromatographic method of ACh assay.

2. Materials and methods

Male albino Holtzman rats of 3 age groups were used in the study; young rats (28 days old), adult rats (approximately 90 days old) and geriatric rats (approximately 2 years old). The rats were housed in groups of 4 and kept on a circadian rhythm of 12 hr dark (7.00 pm–7.00 am) and 12 hr light (7.00 am–7.00 pm). All groups of rats were sacrificed by decapitation 2 hr into the light cycle, 30 min after administration of varying doses of d-amphetamine SO_4 i.p. or equimolar Na_2SO_4 in 0.9% NaCl as control.

Total brain ACh was extracted and assayed by a modification of the method of Szilagyi et al. (1972). Rats were decapitated, each brain minus cerebellum was dissected out, weighed, and placed in a separate homogenizing tube containing a mixture of 9.0 ml acetonitrile (reagent grade), 3.0 ml deionized, distilled H_2O (pH 4.0–5.0) and 25 nmoles propionylcholine iodide (1 nmole/μl solution internal standard). The time from decapitation to homogenization was always less than one min. The brain was homogenized, shaken on ice in a Duboff shaker for 15 min,

and centrifuged for 5 min at 4000 rpm. The supernatant was decanted, the pellet washed with 1 ml of acetonitrile, and the aqueous phase extracted with twice volume of diethyl ether. This solution was shaken for 5 min, centrifuged at low speed, and the ether decanted off. The ether extraction was then repeated. From the final aqueous solution, 0.5 ml was taken and mixed with 0.5 ml water (pH 4.0–5.0), 50 μg tetramethylammonium iodine, and 2.0 ml KI–I_2 solution (2 g of KI and 1.6 g of I_2 in 10 ml of water). This solution was incubated on ice for 30 min, then centrifuged for 20 min at 4800 rpm. The supernatant was removed. The precipitate was dryed and assayed by pyrolysis gas–liquid chromatography using a Hewlett–Packard Model 5750B instrument with 8 ft × 4 mm aluminium columns packed with 60–80 mesh Chromosorb W HMDS coated with 20% Carbowax 6000.

All data were analyzed for statistical significance using Student's *t*-test for group comparison.

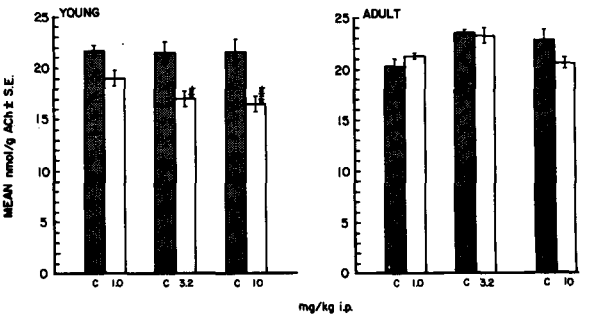

Fig. 1. The effects of varying doses of d-amphetamine on total levels of brain acetylcholine in young and adult rats. The bar graph on the left refers to 28 day old animals and the one on the right to animals approximately 90 days old. The graphs indicate the total brain acetylcholine in nmoles per gram of brain after the administration of various doses of d-amphetamine. The shaded columns represent controls, and the light columns are drug-treated. Each column represents an *n* of 8 animals. Significance was determined by Student's *t*-test (** $p < 0.02$, *** $p < 0.01$).

3. Results

3.1. Steady state brain ACh as a function of age

Steady state brain ACh following injections of Na_2SO_4–NaCl did not vary significantly in young, adult and geriatric animals as shown in the table below.

3.2. Effects of d-amphetamine on brain ACh as a function of age

The effects of d-amphetamine on brain ACh varied depending upon the age of the animals. The data for young adult rats is presented in fig. 1. It is apparent from the figure that d-amphetamine had no effect on brain ACh in the adult rat, even in doses that produce maximum psychomotor stimulation (Schulte et al., 1941). In the young rats, however, d-amphetamine caused a dose-related decrease in brain ACh. In the two year old rats, d-amphetamine decreased brain ACh similar to that seen in young rats. A dose of 2.0 mg/kg i.p. reduced total brain ACh from 20.0 ± 1.1 nmoles/g (controls) to 16.8 ± 0.5 nmoles/g ($n = 8$). This decrease was highly significant ($p < 0.001$). Thus, brain ACh levels appear to change following d-amphetamine in very old and young but not in adult rats.

Table 1
Mean total brain acetylcholine levels in untreated rats of various age groups. Group comparison to young animals determined by Student's *t*-test.

Group	*n*	Mean brain ACh ± S.E. (nmoles/g)	*p Value comparison
Young (28 days old)	19	21.7 ± 0.1	
Adult (90 days old)	18	22.2 ± 1.0	N.S.
Old (24 months old)	8	20.0 ± 1.1	N.S.

*Group comparison to young animals.

4. Discussion

It is well known that d-amphetamine causes the release of ACh from the brain (Bartolini et al., 1971; Beani et al., 1969; Deffeau et al., 1970; and Hemsworth and Neal, 1968). On this basis, one would expect that d-amphetamine would enhance the turnover of brain ACh. Indirect evidence for this has been obtained in the rat using hemicholinium-3, given intraventricularly, and d-amphetamine, given i.p., to the

rat (Domino and Wilson, 1971). If brain ACh synthesis can keep up with its utilization, one would not expect any change in total levels following d-amphetamine. If utilization is greater than synthesis, however, then total levels should decrease. It appears from the results of the present study that in young and very old rats the latter is true, while in adults, the former is true. Recently, it has been shown that in old rats there is a dramatic decrease in the activity of choline acetyltransferase, the enzyme which synthesizes ACh, and AChE, the enzyme that hydrolyzes ACh (Frolkis et al., 1973). It is readily apparent that what appeared to be a discrepancy in the results from different laboratories on the effects of d-amphetamine on total brain ACh is now resolvable based upon the ability of animals to maintain brain ACh synthesis with increased demand. Obviously, additional studies using mice are indicated. The age of the animals, however, is a critical factor investigators must keep in mind when studying drug effects on brain ACh.

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