A DEHYDROGENASE SYSTEM TO DEMONSTRATE OXIDATION OF AMPHETAMINE AND RELATED COMPOUNDS BY RAT BRAIN HOMOGENATE

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Summary: Amphetamine, ephedrine, mescaline and chlorpromazine act as substrate for dehydrogenase system of rat brain homogenate. This system appears quite different from the usual deaminating process of these compounds, which required NADP and oxygen. The present system proceeds more rapidly in anaerobic condition.

Key words: rat brain amphetamine & related compounds dehydrogenation

INTRODUCTION

Information concerning the metabolic degradation of amphetamine and other related compounds like ephedrine, mescaline and chlorpromazine is very little and scanty (1,2). These compounds are now widely used as a stimulant of the central nervous system and its major route of biotransformation in the rat at least involves hydroxylation and deamination. The present report describes an alternate pathway in rat brain which is dehydrogenase in nature and acts more repidly in anaerobic condition.

MATERIALS AND METHODS

Dehydrogenase activity was assayed essentially by the reduction of neotetrazolium chloride (NTC) as described by Ghosh and Guha (3) for the estimation of monoamine dehydrogenase (MADH). In a subsequent experiment this brain homogenate was used an enzyme.

Selection of substrate:

D-amphetamine and its isomer L-amphetamine, mescaline, ephedrine and chlorpromazine were used as substrates. Since tyramine was used as the substarte for the assay of MADH in the original method, tyramine has also been used here as the additive substrate in order to see the capability of formazan production.

Aerobic assay method for dehydrogenase activity:

The reaction mixtures contained 0.025 M phosphate buffer pH 7.0, 0.5 *mg* NTC and 100 *mg* of tissue homogenate. The final volume of reaction mixtures was 2 *ml* and kept in a thermostatic water bath at 38°C for 5 minutes in order to obtain a uniform temperature, then 0.01 M substrate

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(pH adjusted to 7.0) was added. The mixture was further incubated for a period of 30 minutes, where air was used at the gas phase. At the end of incubation, suitable solvent was added to extract the formazan which was formed due to the activity of the enzyme.

Anaerobic assay method for dehydrogenase activity:

Anaerobic experiments were performed *in vacuo* in Thunberg's tube and incubation time was for 15 minutes only. The reaction mixtures remained same as described under aerobic method. All the reaction mixtures except NTC and substrate were placed in the main tube while NTC and substrate were kept in the side arm. The final reaction volume was 2.0 *ml* and the enzyme reaction was proceeded *in vacuo*. Evacuation of the Thunberg's tube was carried out by means of a vacuum oil pump. At first, grease was added on the ground joint of the cap in 90° angle from the hole of the ground joint. The hole in the cap was coincidated with the evacuation outlet of the tube joint. The evacuation outlet was then attached to a vacuum oil pump and evacuated for 3 minutes. When evacuation was complete, the cap was rotated slowly through an angle of 180° and oscillated it through a small arc to set up the cap. After 5 minutes' pre-incubation, contents of the side arm (NTC and substarte) were mixed with the reaction mixtures of the tube by gentle jerks. After 15 min incubation, the formazan was extracted by a suitable solvent.

Extraction of formazan:

After the expiry of the incubation period, 4.0 *ml* butanol was added to extract the formazan which was formed during the incubation period. The mixture was centrifuged to ensure clear and complete separation of the two layers and the butanol layer was then carefully aspirated out. This butanol layer which was red coloured due to formazan extraction was measured in a Bausch & Lomb colorimeter at 520 *mμ*. All values were corrected for suitable blanks which were run simultaneously but containing no substrate. Results were expressed in terms of observed readings of O.D. measured at 520 *mμ*.

RESULTS AND DISCUSSION

D- and L- amphetamine, ephedrine, mescaline and chlorpromazine are acting as good substrates in this system. The rate of dehydrogenation in presence of these substrates is proceeded like tyramine (Table I). When both tyramine and any one of these five compounds are added, they behave like additive substrate though there appears a partial inhibition. It is expected that this inhibition is caused due to high concentration of the substrate (Table II). Furthermore, these compounds stimulate greatly the reduction of NTC in absence of oxygen. Oxygen cannot be replaced by NTC. So, this system appears quite different from the deamination process of the sympathomimetic amines as described previously by Axelrod (1,2), because according to that system, deamination only could proceed in presence of oxygen and nicotinamide adenine

dinucleotide phosphate (NADP). Dehydrogenase process of these drugs by brain tissue is expected to pave a new path in psychiatric therapy because these drugs are popularly used in psychologic disorders.

TABLE I: Showing D- and L- Amphetamine, Mescaline, Ephedrine and Chlorpormazine acting as substrate.

Sl. No.	Substances	Final concentration (M)	MADH (Aerobic) E_{520} Mean ± SD	MADH (Anaerobic)* E_{520} Mean ± SD
1.	D-Amphetamine	1 x 10^{-2}	0.260 ± 0.027 (5.42 ± 0.56)	0.230 ± 0.021 (9.58 ± 0.88)
2.	L-Amphetamine	1 x 10^{-2}	0.220 ± 0.042 (4.58 ± 0.85)	0.220 ± 0.033 (9.16 ± 1.38)
3.	Mescaline	1 x 10^{-2}	0.240 ± 0.020 (5.00 ± 0.42)	0.240 ± 0.013 (10.00 ± 0.54)
4.	Ephedrine	1 x 10^{-2}	0.210 ± 2.20 (4.38 ± 0.42)	0.220 ± 0.025 (9.16 ± 1.04)
5.	Chlorpromazine	1 x 10^{-3}	0.210 ± 0.021 (4.38 ± 0.44	0.270 ± 0.013 (11.26 ± 0.54)

Figures in parentheses represent μmoles of diformazan formed/min.
* NTC reduction was measured in these experiments after 15 min incubation.

TABLE II: Showing D- and L- Amphetamine, Mescaline, Ephedrine and Chlorpromazine acting as additive substrate (in presence of tyramine (1 x 10^{-2} M).

Sl. No.	Substances	Final concentration (M)	MADH (Aerobic) E_{520} Mean ± S.D.	MADH (Anaerobic)* E_{520} Mean ± S.D.
1.	D-Amphetamine	1 x 10^{-2}	0.400 ± 0.077 (8.33 ± 1.60)	0.290 ± 0.012 (12.08 ± 0.50)
2.	L-Amphetamine	1 x 10^{-2}	0.380 ± 0.048 (7.92 ± 1.00)	0.390 ± 0.050 (16.26 ± 2.08)
3.	Mescaline	1 x 10^{-2}	0.260 ± 0.022 (5.42 ± 0.46)	0.290 ± 0.016 (12.08 ± 0.66)
4.	Ephedrine	1 x 10^{-2}	0.240 ± 0.021 (5.00 ± 0.44)	0.370 ± 0.021 (15.42 ± 0.88)
5.	Chlorpromazine	1 x 10^{-3}	0.380 ± 0.041 (7.92 ± 0.85)	0.430 ± 0.030 (17.92 ± 1.26)

Figures in parentheses represent μmoles of diformazan formed/min.
* NTC reduction was measured in these experiments after 15 min incubation.

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