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The Effects of d-Amphetamine on the Brain 5-HT, 5-HIAA, MAO and the Behavior of Rat

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=국문초록=

d-Amphetamine이 백서뇌의 5-HT, 5-HIAA, MAO 및 행동에 미치는 영향에 관한 연구

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d-amphetamine이 사람에서 paranoid schizophrenia와 아주 유사한 model psychosis를 일으키며 또한 사람과 실험동물에서 실제 정신분열증에서 뚜렷이 관찰되는 behavioral perservation을 일으킬 수 있음이 관찰되었다. 이에 많은 학자들은 이러한 양상의 행동변화가 정신분열증의 원인 추구에 중요한 의미를 주는 뇌변화를 반영할지도 모른다는 생각에 많은 연구를 거듭하여 왔다.

지금까지는 주로 catecholamine 기전에 대하여 집중적 연구가 수행되어져 왔으나 최근에는 d-amphetamine의 약리기전의 일부는 5-HT 기전이 차지하고 있으며, 여러 행동변화에는 catecholamine보다 5-HT가 더 중요하게 관계하고 있다는 주장이 나오고 있다.

또한 d-amphetamine은 시험관내에서 MAO 특히 신경전달물질 분해효소인 A type를 가역적으로 억제할 수 있음이 보고되어 많은 흥미를 끌어왔으나 생체내에서의 억제여부는 직접적으로 확인이 되고 있지 않다. 그러나 최근에 Braestrup(1977)과 El Hait(1978)등은 간접적인 방법으로 생체내에서도 억제시킬 수 있음을 보고하고 있다.

이에 저자는 d-amphetamine에 의해 야기되는 행동변화와 그 밑바탕을 이루는 생화학적 기전에 5-HT가 차지하는 역할을 알아보기 위해서 다음의 실험을 시행하였다.

첫째, d-amphetamine의 급성, 만성 투여가 5-HT의 5-HIAA로의 turnover와 MAO활성도에 어떤 영향을 미치며, 더 나아가서 이 양자사이에 어느 정도 상관관계가 있는지를 알아보기 위해서 d-amphetamine을 투여한 후 시간 경과에 따라서 뇌내 5-HT, 5-HIAA, 5-HT turnover rate와 MAO활성도를 측정하였다.

둘째, d-amphetamine, 5-HT 합성을 증가시키는 약물과 합성을 억제시키는 약물을 투여하고, 위의 생화학적 실험과 행동관찰을 병합 실시하여 비교분석하였다.

그 결과는 다음과 같다.

1) d-amphetamine (6 mg/kg)을 급성투여시, 뇌내 5-HT 함량이 투여 1시간 후에 최고로(대조치의 123%, $p<0.001$) 증가되다가 이후 감소하며, 5-HIAA 함량은 처음 15분부터 감소하기 시작하다가 30분에 최저로 떨어지며(대조치의 78%, $p<0.005$) 이후 증가하여 24시간째는 약간 대조치 이상으로 회복되었다. 미토콘드리아 MAO활성도는 1시간째에 최저로 떨어지다가(대조치의 89%, $p<0.05$) 이후 회복하기 시작하여 24시간째에 약간 대조치 이상으로 회복되었다. 5-HT의 turnover rate

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는 MAO활성도 변화와 거의 같은 변화를 보였다.

2) 만성투여시(하루 2번, 14일간 투여)는 5-HT함량, 5-HIAA함량, MAO활성도 및 5-HT turnover rate모두가 중등도로 감소되었다. (각각 대조치의 87%, 69%, 80%, 79%).

3) MAO활성도와 5-HT turnover rate사이에는 높은 상관관계가 있었다($r=0.866$, $p<0.001$, $N=94$).

4) MAO활성도의 역동학 실험에서는 대조치에 비해 투여군에서 Km값은 의미있는 증가가 있었으나 V_{max}값은 큰 변동이 없었다.

5) d-amphetamine을 급성 투여할때는 sleeping과 lying components는 상당한 감소를 보인 반면, locomotor activity는 1시간까지는 상당한 증가를 보였으며 용량이 적을수록 더 큰 증가가 있었다. 반면 stereotypy는 1시간까지 용량이 증가할수록 더 큰 증가가 나타나서 locomotor activity에서 stereotypy의 증가로 이행을 나타내었다. 만성 투여시는 locomotor activity는 점차적인 감소를 보였으나 stereotypy는 점차적인 증가가 나타나서 14일쯤에는 평형에 도달하였다.

6) PCPA 단독 투여군(400 mg/kg, 3번)에 있어서는 5-HT와 5-HIAA함량의 상당한 감소가 나타났으나 MAO활성도와 행동에는 큰 변화를 나타내지 않았다. PCPA전 처치군에 있어서도 5-HT와 5-HIAA함량은 마찬가지로 상당한 감소를 나타내었으나 gnawing, sniffing과 locomotor activity는 더 증가를, stereotyped head weaving, forepaw treading과 hindlimb abduction은 상당한 감소를 나타내었다.

7) L-tryptophan(100 mg/kg)단독 투여시는 5-HT함량은 약간 증가를 나타내었으나 5-HIAA함량은 상당한 증가를 보였다. MAO활성도나 행동은 큰 변화없었다. L-tryptophan 전처치군에 있어서는 5-HT함량은 더 큰 증가를 보였으나, 5-HIAA함량과 MAO활성도는 별 변화없었으며 stereotyped lateral head weaving, forepaw treading과 hindlimb abduction은 더 증가를, locomotor activity, gnawing과 sniffing components는 감소를 나타내었다.

8) d-amphetamine 단독투여, 혹은 L-tryptophan전처치, PCPA전처치후 측정한 5-HT함량과 stereotyped head weaving, forepaw treading, hindlimb abduction components사이에는 높은 상관관계가 있었다($r=0.789$, $p<0.001$). 반면 5-HT함량과 locomotor activity, stereotyped gnawing과 sniffing components사이에는 약한 음성의 상관관계가 있었다($r=0.554$, $p<0.005$).

이상의 결과로 미루어 볼 때 5-HT의 5-HIAA로의 turnover rate는 주로 MAO활성도에 의해서 조절이 되며 5-HT기전이 d-amphetamine에 의해서 야기된 여러 행동변화중 상당한 부분에서 중요한 역할을 하리라고 생각된다.

Amphetamine causes a variety of CNS stimulating effects and various behavioral changes in men and animals, which were first described by Piness (1930) and Alles et al (1933). It is well known that amphetamine induces psychosis so closely mimicking paranoid schizophrenia that amphetamine intoxication must be considered in the differential diagnosis of any patients with signs of this disorder. There appears to be a general consensus that behavioral perseveration is a fundamental feature of the amphetamine response in both experimental animals and human and is also apparent in at least some forms of naturally occurring schizophrenia. It has been assumed that amphetamine induced behavioral perserveration may reflect a CNS alteration that is etiologically significant in schizophrenia and so amphetamine has frequently been used as a research tool for the study of biochemical basis of schizophrenia.

Many investigators have generally believed that amphetamine was a prototype activator of catecholamine mechanisms in the CNS, but more recent findings strongly suggest that 5-HT mechanisms are involved in, at least, some of the effects of d-amphetamine and moreover, play a more important role in the expression of some of behavioral effects than catecholamine mechanisms(Grahame-Smith, 1971; Squires and Lassen, 1975; Jacobs,

1976; Sloviter et al., 1978; Deakin and Green, 1978; Gerson and Baldessarini, 1980). In spite of extensive studies during the last two decades, the functional role of the 5-HT neurons is still unclear.

Also, d-amphetamine has been known to be a reversible inhibitor of MAO in vitro, with a preference for MAO type A which metabolizes the transmitter amines including 5-HT (Mann and Quastel, 1940; Blaschko and Pratesi, 1963; Green, 1971; Mantle et al., 1976) but there is no considerable evidence for in-vivo inhibition.

Objectives of the present study are: 1) to determine if, and to what extent, the turnover of 5-HT and MAO activity are influenced by d-amphetamine, and furthermore, to search for correlations between changes in MAO activity and in the rate of 5-HT turnover in the CNS and 2) to determine whether some of the effects of d-amphetamine on behavior can be ascribed to actions of the drug on 5-HT mechanisms.

Materials and Experimental Methods

Materials

Animal: Male sprague-Dawley rats (SNU Laboratory animal quarter, Seoul) weighing 180 to 240 g were used in all experiments. Animals were housed in a quiet room to a cage (20 cm×20 cm×20 cm) under a 12hr light-dark schedule at 25±5°C. Food and water were available ad libitum. Control and experimental groups were treated identically except that control rats were injected with saline. Experiments were performed at 15 min, 30 min, 1 hr, 2 hr, 4 hr and 24 hr after injection of d-amphetamine (6 mg/kg), but times for sacrifice were chosen between 11 : 00~12 : 00 A.M.

Drugs and Reagents

d-Amphetamine sulfate (Sigma), p-Chlorophenylalanine (PCPA, Sigma) and L-Tryptophan (Merck) were dissolved in 0.9% NaCl solution and injected 2.0 ml/kg body w.t. intraperitoneally. 5-Hydroxytryptamine creatinine sulfate (5-HT), 5-Hydroxyindole 3-acetic acid (5-HIAA), Kynuramine dihydrobromide, 4-Hydroxy quinoline, Glucose 6-phosphate, and 5-AMP were purchased from Sigma Chemical Co. St. Louis, Mo. U.S.A.. All the common laboratory chemicals employed were reagent grade obtained from Sigma Chemical Co., Shinyo Pure Chemical Co., or Shimakyu's Pure Chemical Co. Batches of 500 ml n-butanol were washed successively with 50 ml 1N NaOH, 50 ml 1N HCl and 4×50 ml water in a separating funnel, then saturated with NaCl. Heptane was washed successively with (1N NaOH, distilled water, 1N HCl, distilled water), twice. 0.004% w/v o-phthalaldehyde (OPT) in 10 N HCl, 0.1% w/v cysteine in 0.1 N HCl 1% cysteine in deionized water, and 0.1% w/v OPT in methanol were all prepared immediately before use.

Methods

1) **5-HT and 5-HIAA Analysis**: Rats were killed by guillotine. After decapitation, brains were rapidly removed from the calvaria and placed on watch glass cooled on ice. A midsagittal cut from the rostral pole to the caudal medulla divided the brain into right and left halves. The right half was taken for 5-HT and 5-HIAA assays of intact brain by the method of Curzon and Green (1970). The other half was enclosed immediately in plastic vals and frozen on dry ice and stored at −20°C until the analysis of MAO activity. All tissues were assayed within 2 days.

Frozen brain tissues were homogenized in 10 volumes of cold acidified n-butanol with a Potter Elvehjem tissue grinder (clearance 0.006~0.009). After centrifugation for 5 min at 600 g, 8.0 ml of the supernatant was pipetted into a 50 ml glass stoppered tube and shaken mechanically for 10 min with 5 ml n-heptane and 1.5 ml 0.1 N HCl containing 0.1% cysteine. The phases were separated by centrifugation as before and 10 ml of the organic phase retained for the 5-HIAA assay.

To determine 5-HT, 0.5 ml aliquots of the aqueous phase were pipetted into test tubes and 3 ml of 0.004% OPT in 10 N HCl added. After mixing with a rota mixer and heating in a boiling water

bath for 15 min, the tubes were cooled in water and fluorescence measured using Perkin-Elmer Model 1000 Spectrofluorimeter. Activation and fluorescent wavelengths were 360 nm and 470 nm (both uncorrected) respectively. Standards were prepared as 40 μg/ml solutions in deionized water and stored at −20°C. Tissue blanks were prepared by reacting 0.5 ml of the aqueous phase with 1.8 ml 10 N HCl. Calculations were made with reference to the value of the internal standard which is carried through the entire estimation procedure.

To determine 5-HIAA, the 10 ml of organic phase remaining after the extraction of 5-HT was pipetted into a 25 ml glass stoppered tube containing 2 ml 0.5 M phosphate buffer (pH 7.0) and shaken mechanically for 10 minutes. After centrifuging for 3 min at 600 g, two 1 ml aliquots of the aqueous phase were pipetted into two test tubes, A and B. To A was added 0.1 ml 1% cysteine solution and to B 0.2 ml of 0.02% sodium periodate solution. Then 2 ml concentrated HCl was added to both A and B. After then 0.2 ml of OPT solution (0.1% in methanol) and 0.2 ml periodate solution were added to tube A. After 30 min, 0.1 ml of cysteine and OPT solutions were added to tube B. The tubes were then placed in a boiling water bath for 10 min, cooled in water and read at activation: 360 nm, fluorescence: 470 nm (both uncorrected). The blank reading (tube B) was subtracted from the test reading (Tube A). Standards were prepared as 40 μg/ml solutions and stored at −20°C. Average recovery of 5-HT and 5-HIAA was 86% and 95% respectively.

2) Assays of MAO activity:

① Isolation of brain mitochondria and analysis of its purity.

The brains were homogenized in 9 volumes (w/v) of 0.25 M sucrose in a Potter Elvehjem homogenizer at 0°C for 30 sec with 5 pestle strokes by the modified method of Brody and Bain (1952). The homogenates were centrifuged at 600g for 10 min in a refrigerated RC-2 Sorvall centrifuge. The sediment was discarded and the supernatant fluid was centrifuge at 10,000 g for 20 min. The crude mitochondrial fractions were washed, defatted, resuspended in the buffered sucrose, and centrifuged at 16,000 g for 20 min. Above procedures were repeated. After carefully decanting the supernatant fluid, the mitochondrial pellet was suspended in homogenation medium (ca. 1 mg protein/ml) and was used for the assay. The assessment of the purity of the brain mitochondrial fractions was done through the use of enzyme marker studies and electron microscopic examination. Enzyme marker studies included 5-nucleotidase (plasma membrane marker) by the method of Segal and Brenner (1960), G-6-phosphatase (microsomal marker) by the method of Swanson (1965) and Grinna and Barber (1972), and MAO

Table 1. Effects of acute and chronic d-amphetamine administration on 5-HT, and 5-HIAA of rat brain (Unit: μg/g)

Groups	Time after injection	No.	5-HT		5-HIAA	
			Mean±S.D.	P value	Mean±S.D.	P value
Control		18	0.536±0.084	N.S	0.377±0.063	N.S
Acute	15 min.	12	0.570±0.062	N.S	0.318±0.090	N.S
	30 min.	10	0.594±0.066	0.05	0.295±0.060	0.005
	1 hr.	11	0.660±0.066	0.001	0.337±0.040	0.05
	2 hr.	11	0.627±0.099	0.02	0.387±0.112	N.S
	4 hr.	14	0.572±0.052	N.S	0.402±0.060	N.S
	24 hr.	10	0.538±0.095	N.S	0.442±0.060	0.01
Chronic		10	0.468±0.028	0.005	0.259±0.016	0.005

N.S.: non significant.

Table 2. Enzyme activities of marker enzymes in 10% homogenate and in intact mitochondrial fraction in rat brain

Organelle	Marker enzyme	Specific activity in mitochondrial fraction/homogenate	Relative activity
Plasma membrane	5′-nucleotidase	*0.453/1.530	0.296
Microsome	Glucose-6-phosphatase	*0.208/0.495	0.420
Mitochondria	MAO	**0.192/0.061	3.20

Each value is given as a mean from triplicate determinations of the sample from the pooled brains of 5 rats.
*: μmoles Pi/hr/mg protein.
**: μmoles 4-hydroxyquinoline/hr/mg protein.

(mitochodrial marker) by the method of Kraml (1965). The mitochondrial fraction seemed to be reasonably free of contamination by other subcellular organelles and appeared to have a fair degree of morphological integrity(Table 2).

② Activity assays

MAO activity with kynuramine was determined by measuring 4-hydroxyquinoline production using a modification of the spectrofluorometric method described by Kraml(1965). The standard assay mixture, consisting of 50 mM potassium phosphate buffer, pH 7.4, 0.1 mM kynuramine, and 50~100 μg enzyme in a final volume of 2.0 ml, incubated at 37°C for 20 min. The reaction was started with addition of substrate and stopped by the addition of 0.6 M perchloric acid. The fluorescence was measured at an emission wavelength of 410 nm, excited at 312 nm.

The kinetic constants, Km and V_{max} for the substrate kynuramine of MAO were determined with the standard assay procedures as described above. The constants were derived from double reciprocal plots as described by Lineweaver & Burk(1934). Linear graphs were obtained and the line of best fit was determined by the method of least squares. Correlation analysis was performed. The correlation coefficient(r) for all of the kinetic data plots were 0.90 or greater. Two or seven kinetic determinations were perfo-rmed for each constant. For determination of the kinetic constants, a concentration range of 2×10^{-5} to 1×10^{-3} M was used.

Protein was determined by the method of Lowry et al(1951), using bovine serum albumin as standard.

3) Behavioral Experiments

Rats were removed from the cages only for injections and sacrifice. From injection until sacrifice rats were observed continually by two investigatiors. The behaviors were evaluated in all-or-none-manner. Each animal was observed ten times at 15 min, 30 min, 60 min, 120 min, 240 min, and 24 hr after injection(2 mg/kg & 6 mg/kg) in acute study, and each rat was observed ten times 1 hr after injection every day in chronic study. Each of the signs was scored in each rat 10 times at a observation time(absent: 0, present: 1). Cumulative scores for each rat were calculated at the end of observation period and expressed as total mean score for the group(maximally 10 points for each behavioral item). Observed behavioral components consist of sleeping and lying, rearing and locomotor activity, five stereotyped behaviors (gnawing, sniffing, lateral head weaving, forepaw treading and hindlimb abduction).

4) Statistical Analysis

Levels of significance were calculated by student's t-test or Mann-Whitney U test(two tailed test). Significant deviation from control had a probability less than 0.05.

Correlation analysis was performed.

Linear graphs were obtained and the line of best fit was determined by the method of least squares.

Table 3. Effects of d-amphetamine on mitochondrial MAO activity and 5-HT turnover rate of rat brain

Groups	Time after injection	No	Mitochondrial MAO activity (mol/mg/hr)		5-HIAA/5-HT		Correlation coefficient	
			mean±S.D.	P	mean±S.D.	P	r	p
Control		21	0.189±0.002	N.S	0.703±0.197	N.S	0.798	0.001
Acute	15 min	9	0.177±0.021	N.S	0.558±0.090	0.005	0.843	0.01
	30 min	13	0.176±0.007	0.05	0.497±0.072	0.001	0.872	0.001
	1 hr	9	0.169±0.021	0.05	0.510±0.036	0.001	0.896	0.005
	2 hr	10	0.180±0.006	N.S	0.617±0.079	N.S	0.857	0.005
	4 hr	9	0.185±0.021	N.S	0.703±0.105	N.S	0.795	0.02
	24 hr	13	0.218±0.036	0.05	0.822±0.230	N.S	0.891	0.001
Chronic		10	0.152±0.025	0.001	0.553±0.028	0.01	0.932	0.001
							0.866	0.001

Results

1) Effects of d-amphetamine on brain 5-HT, 5-HIAA, 5-HT turnover, and MAO activity

5-HT concentration rose to the maximum in 1 hr (123% of control, $p<0.001$) and then declined by d-amphetamine, single injection but 5-HIAA level began to decrease at 15 min post injection, reaching lowest level at 30 min(78% of control, $p<0.005$) and returned to control level but slightly increased at 24 hr after injection.(Table 1 and Fig. 2).

While, MAO activity declined to a minimum at 1 hr(89% of control, $p<0.05$) and then returned to control level at 4 hr but slightly increased at 24 hr post injection(Table 3 and Fig. 2).

5-HT turnover rates(5-HIAA)/5-HT) revealed almost nearly same changes as MAO activity (Table 3 and Fig. 2).

The results presented in Table 1 and Fig. 2 illustrated that chronic administration of d-amphetamine(6 mg/kg) twice a day for 14 days produced a moderate decrease in 5-HT level, 5-HIAA level, MAO activity and in 5-HT turnover rate(87%, 69%, 80%, 79%, of controls, respectively).

The good correlation between MAO activity and the 5-HT turnover rate was evidenced in Table 3 and Fig. 1($r=0.866$, $p<0.001$, $N=94$).

Table 4. The *In-vivo* effect of d-amphetamine on kinetic constants for MAO in rat brain mitochondria

Groups	Time after injection	km (μm)	V_{max} (μmol/mg/hr)
Control		32.0±2.3	0.228±0.007
Acute	15 min	41.7+2.7**	0.228±0.010
	30 min	43.8±4.5*	0.236±0.010
	1 hr.	49.7±5.2**	0.224±0.014
	2 hr.	40.4±4.1	0.233±0.009
	4 hr.	37.5±2.7	0.235±0.009
	24 hr.	32.6±2.1	0.264±0.011
Chronic	1	43.6±3.4**	0.203±0.015

Mean±S.E.M. from duplicate determinations of the sample from 6 to 8 rats.
*: $p<0.05$ **: $p<0.02$ ***: $p<0.005$

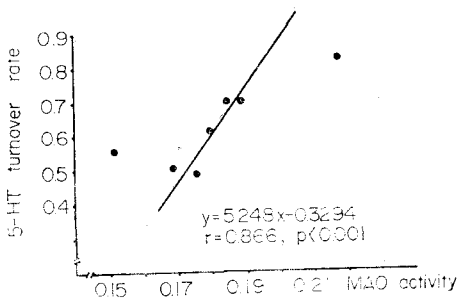

Fig. 1. Correlation between MAO activty and 5-HT turnover rate in rat brain.

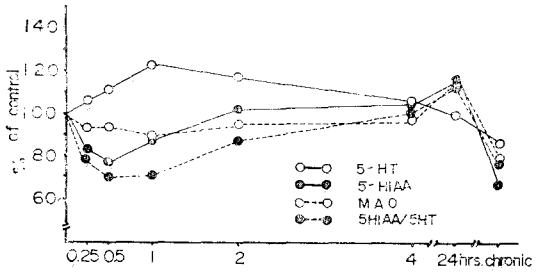

Fig. 2. Time course of the acute and chronic effects of d-amphetamine on 5-HT, 5-HIAA, 5-HT turnover and MAO activity in rat brain.

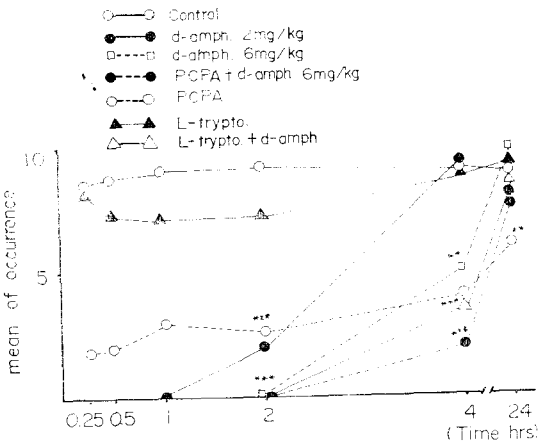

Fig. 3. Acute effect of d-amphetamine on the sleeping and lying components in rat.

*<p 0.05 Significant differences from control Gp.

**<p 0.02

***<p 0.002

The kinetics of MAO showed a moderate and statistically significant increase in the Km but no significant changes in the V_{max} after injection of d-amphetamine (Table 4).

2) Effect of d-amphetamine on behavior

Acute administration of d-amphetamine markedly decreased the number of sleeping and lying component (Fig. 3). In contrast, locomotor activity and rearing components were initially increased and then decreased in a dose reversed fashion, but stereotypy as a whole was gradually increased to 1 hr after injection in a dose related manner (Fig. 4 and 5).

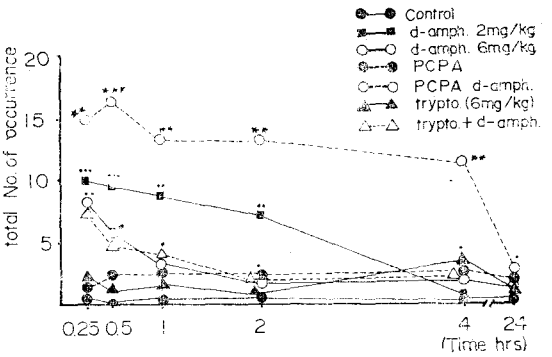

Fig. 4. Acute effect of d-amphetamine on the walking and rearing components in rat.

* p<0.05 * Compared with control Gp.

** p<0.02 • Compared with d-amph (6 mg/kg) Gp.

*** p<0.002

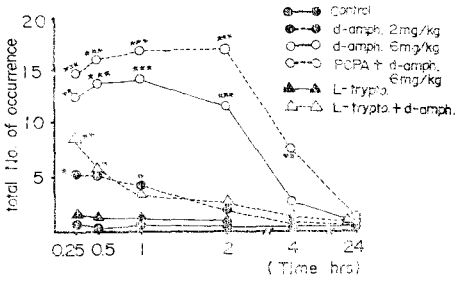

Fig. 5. Acute effect of d-amphetamine on the stereotypy in rat.

* p<0.05 Compared with control Gp.

** p<0.01

*** p<0.001

Chronic administration of d-amphetamine twice a day produced gradual decrease in locomotor activity in a dose reversed fashion, but gradual increase in stereotypy in a dose related manner for 14 days and then plateau level reached (Fig. 8, 9, 10).

3) Effects of PCPA and L-tryptophan pretreatment

PCPA given alone (three 400 mg/kg doses) induced a marked depletion of the concentration of 5-HT and of 5-HIAA without significant effect on MAO activity and was largely without significant effects on motor activity and behavior (Table 5,

Table 5. Effects of PCPA-and tryptophan-pretreatment on rat brain 5-HT, 5-HIAA and MAO measured 60 *min* after d-amphetamine injection

(Mean±S.D.)

Groups	No.	5-HT	5-HIAA	MAO	5-HIAA/5-HT
Control	8	0.534±0.051	0.379±0.060	0.189±0.017	0.703±0.122
PCPA	6	0.111±0.019***	0.056±0.006***	0.140±0.232	0.505±0.262
Tryptophan	6	0.538±0.044	0.502±0.039***	0.192±0.062	0.935±0.161**
d-amphetamine	11	0.662±0.072***	0.337±0.040*	0.169±0.021*	0.510±0.042***
+PCPA	6	0.124±0.042***,+++	0.047±0.031***,+++	0.112±0.100*	0.389±0.198**
+L-trypto.	10	0.797±0.028***+++	0.355±0.031	0.163±0.029*	0.445±0.112***

*,+: $p<0.05$ * Compared to controls group
**,++: $p<0.01$ + Compared to d-amphetamine group
***,+++: $p<0.001$

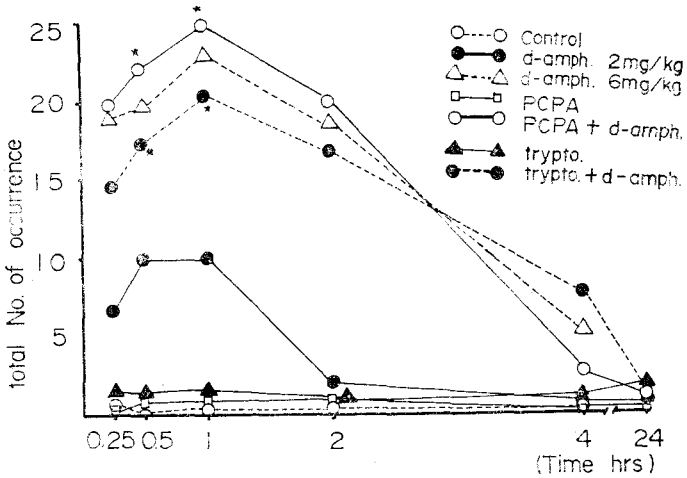

Fig. 6. Effects of d-amphetamine on the stereotyped gnawing and sniffing components in rat.
* $p<0.05$ Compared with d-amphe(6 mg/kg) Gp.
** $p<0.01$

Fig. 3-7). Pretreatment of rats with PCPA for 3 consecutive days at 72,48, 24 hr before giving d-amphetamine(6 mg/kg) reduced brain concentrations of 5-HT to 23.1% and 5-HIAA to 12.5% of control ($p<0.001$) and caused more intense gnawing, sniffing and hyperactivity, but partially antagonized stereotyped components of lateral head weaving, forepaw treading and hindlimb abduction (Table 5, Fig. 3,4,6,7).

When L-tryptophan alone(100 mg/kg) was administered, there was a slight increase in brain 5-HT concentration, but a large rise in 5-HIAA and in 5-HT turnover rate without effect on MAO activity. No gross behavioral changes were occured Table 5, Fig. 3-7). Pretreatment with L-tryptophan 30 min before giving d-amphetamine resulted in a higher concentration of 5-HT and a more pronounced stimulation of stereotyped components of lateral head weaving, forepaw treading and a less stimulation of locomotor activity, gnawing and sniffing components than were obtained after d-ampetamine alone(Table 5, Fig. 4,6,7).

There is a strong positive correlation between the levels of 5-HT measured 1 hr after injection of d-amphetamine alone or d-amphetamine after PCPA or L-tryptophan pretreatment and the

numbers of occurrence of the stereotyped components of head weaving, forepaw treading and hindlimb abduction ($r=0.789$, $p<0.001$, Fig. 11). In contrast, there is a weak negative correlation between the levels of 5-HT and the numbers of occurrence of the stereotyped gnawing and sniffing and of hyperactivity components ($r=0.554$, $p<0.005$, Fig. 12).

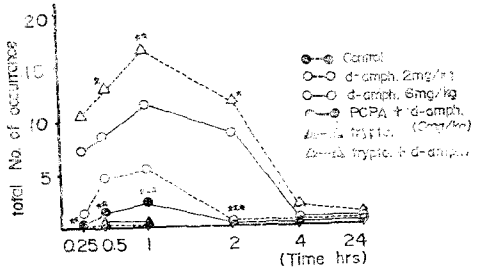

Fig. 7. Acute effects of d-amphetamine on the stereotyped head weaving, forepaw treading and hindlimb abduction in rat.
* $p<0.05$ Compared with d-amphe (6mg/kg) Gp.
** $p<0.01$
*** $p<0.001$

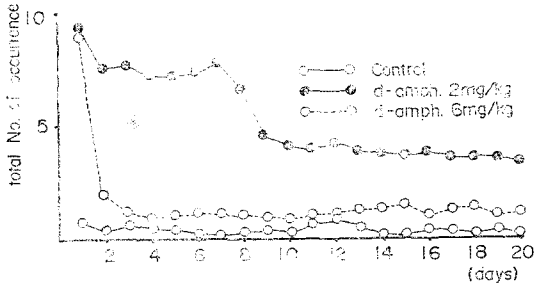

Fig. 8. Effects of chronic d-amphetamine administration on the walking and rearing components in rat.

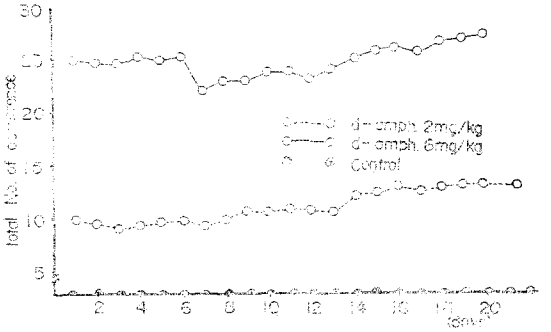

Fig. 9. Chronic effects of d-amphetamine on the stereotypy behavior in rat.

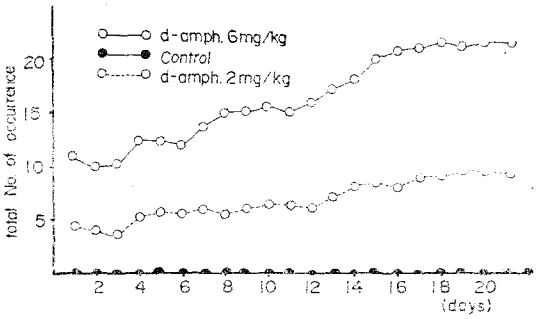

Fig. 10. Chronic effects of d-amphetamine on the stereotyped head weaving, forepaw treading, and hindlimb abduction components in rat.

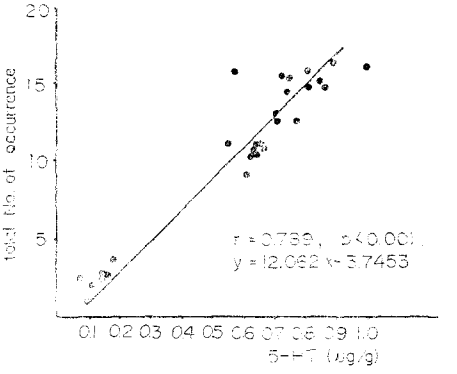

Fig. 11. Correlation between the 5-HT levels caused by d-amphetamine, L-trypto.-or PCPA-pretreatment and the No. of occurrence of stereotyped head weaving, forepaw treading and hindlimb abduction in rat.

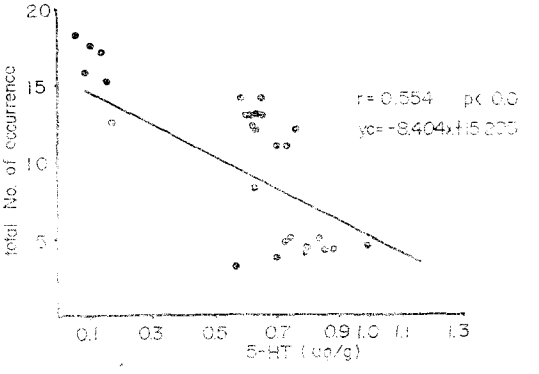

Fig. 12. Correlation between the 5-HT levels caused by d-amphetamine, L-tryptophan or PCPA pretreatment and the No. of occurrence of locomotor activity and stereotyped gnawing and sniffing components in rat.

Discussion

Amphetamine is a unique drug with respect to the simplicity of its structure and the multiplicity of its biological effects. Pharmacologically, amphetamine possesses central stimulant, anorexic, vasoconstrictive and hyperthermic properties. Clinically, amphetamine has been used as a CNS stimulant, antidepressant and appetite supressant, but with repeated administration tolerance to many of its effect develops easily. Amphetamine administered chronically in increasing doses may produce severe symptoms of paranoid psychosis within as short a time interval as five days(Chase and Murphy, 1973; Moore, 1978; Fuller, 1980).

It has been generally believed that amphetamine was a prototype activator of catecholamine mechanisms which involved(1) the inhibition of the reuptake of dopamine & norepinephrine(Glowinski and Axelrod, 1965; Häggendal and Hamberger, 1967; Dengler et al., 1961; Ferris et al., 1972), (2) the release of dopamine & norepinephrine (Stein, 1964; Carlsson et al., 1965; Carr et al., 1970; Ng et al., 1970; Azzaro et al., 1974; Ziance et al., 1972; Rutledge, 1978), (3) the direct stimulation of dopaminergic receptors(Smith, 1963), (4) the inhibition of MAO(Mann and Quastel, 1940; Blaschko and Pratesi, 1963, Mantle et al., 1976; Braestrup, 1977; Clarke et al., 1979).

No other compound has displayed such a plethora of pharmacological, biochemical, and physiological effects, which have endowed this drug with a variety of therapeutic applications and made it a highly effective tool for the pharmacologist and biochemist in the study of the role of neurotransmitter amines in the control of the emotional state and the chemical etiology of certain mental disorders.

Its biochemical properties of releasing catecholamine and inhibiting the cellular uptake of catecholamine coupled with its ability to produce stereotypical behavior in animals have given rise to the plausible hypothesis that the neurotransmitter catecholamine may be implicated in the chemical genesis of psychosis.

The sum of evidence indicates clearly that when amphetamine is used as a research tool, it can be assumed that all actions of the drug are not accounted for by catecholamine mechanisms, and that 5-HT mechanisms are involved in, at least, some of the effects of d-amphetamine and moreover, play more important roles in the genesis of some behavioral effects than catecholamine mechanisms (Grahame-Smith, 1971[a,b], Breese et al, 1974; Jacobs, 1974, 1976; Sloviter et al., 1978; Gerson and Baldessarini, 1980).

5-HT is chiefly catabolized at both inside and outside of the 5-HT neurons via MAO to 5-hydroxyindole-3-acetaldehyde which in turn is converted to 5-HIAA by an aldehyde dehydrogenase.

MAO is widespread in peripheral tissues and in the CNS, where it is intracellulary bound to external membrane of mitochondria (Rodriguez et al., 1962; Schneitman and Greenawalt, 1967). Both glial cells and nerve cells contain MAO(Tipton, 1973). The current evidence indicates that, in many species, the enzyme can be regarded as being composed of 2 major forms which were termed the A and B species by Johnston(1968) according to their sensitivities to inhibition and substrate specificities. Type A is responsible for oxidative deamination of transmitter amines, 5-HT and norepinephrine and inhibited by low concentration of clorgyline(10^{-7}M) and type B deaminates benzylamine and is inhibited by 10^{-5}M deprenil. However, tyramine, tryptamine, dopamine and kynuramine are catalyzed by both types of MAO (Youdim and Collins, 1969; Rutledge et al., 1972; Achee et al., 1974; Gabay et al., 1976; Fowler and Callingham, 1978). But the nature of the two forms of the enzyme has been the subject of considerable speculation(Neff and Yang, 1974). It has been suggested that they may represent two different enzymes, but attempts to separate them in such a pure state that their protein structures could be compared, have been unsuccessful. The possibility that the two forms of the enzyme could

result from the presence of two different active sites on the same enzyme molecule has also been considered(Mantle et al., 1975; white and Wu, 1975). An alternative suggestion is that the two forms could result from modifications of the properties of a single enzyme by its membrane-bound environment(Houslay and Tipton, 1973; Tipton et al., 1973). A considerable amount of evidence has been educed to show the properties of the enzyme are indeed affected by its lipid environment(Houslay and Tipton, 1973; Tipton et al., 1973; Mantle et al., 1976; Fowler and Callingham, 1978; Corte and Tipton, 1979).

Substitution of a methyl group at the α-position of substrates for MAO produces reversible inhibitors which are selective for the A species in vitro(Mantle et al., 1976). In vitro, d-amphetamine is a reversible competitive inhibitor of MAO with a preference for MAO type A(Mann and Quastel, 1940; Blaschko and Pratesi, 1963; Glowinski and Baldessarini, 1966; Green, 1971; Mantle et al., 1976). The selectivity of amphetamine inhibition towards A species is of interest since this species has been shown to be responsible for the oxidation of NE as well as 5-HT.

However, there is no direct evidence for in-vivo inhibition. Recent studies by Braestrup(1977) and Green and El Hait(1978) suggest by indirect confirmation that MAO inhibition may occur in vivo. Our previous results suggested in-vivo inhibition. Our results clearly indicate that d-amphetamine is able to inhibit directly MAO in vivo with a maximal inhibition at 1 hr post injection and the kinetics of in-vivo inhibition reveal competitive inhibition like that of in vitro.

The good correlation between MAO activity and the 5-HT turnover rate was evidenced in our experiments and the results strongly suggest that the 5-HT turnover to 5-HIAA may be chiefly regulated by MAO activity.

Reports on the effects of amphetamine on the biochemical measures of 5-HT neurons in the CNS are inconsistent, few, but studies of time-course are not available except our previous studies. Endogenous brain concentrations of 5-HT have been reported to be increased(McLean and McCartney, 1961; Smith, 1965; Sloviter et al., 1978) or decreased(Laverty, 1965). Some of these differences may be related to strain differences, the magnitude of the dose of the drug or the time after administration that the brains were analyzed.

Possible mechanisms of early rise in 5-HT concentrations in our experiments involve (1) the release of 5-HT(Carlsson et al., 1965; Ng et al., 1970; Rutledge et al., 1972; Azzaro et al., 1973; Sparber and Tilson, 1972; Chiueh and Moore, 1976), (2) the inhibition of reuptake of 5-HT (Häggendal et al., 1967; Wong et al., 1973) and (3) the inhibition of MAO. Trendelenburg and Draskoczy(1972) reported that inhibition of MAO in nerve endings might result in inhibition of the reuptake process as a consequence of rising levels of amines. As stated above, our results suggest that the inhibition of MAO plays a role in the rise of 5-HT concentrations of whole brain.

The synthesis of 5-HT begins with the hydroxylation of L-tryptophan to 5-hydroxyptophan which in turn is decarboxylated to 5-HT. The conversion of L-tryptophan to L-5HTP, catalysed by the enzyme, L-tryptophan hydroxylase, is considered to be rate-limiting. The tryptophan hydroxylase is not normally saturated with tryptophan. Consequently, the rate of 5-HT synthesis depends both on the concentration of tryptophan in 5-HT neurons, which is dependent on the plasma concentrations and on the activity of tryptophan hydroxylase(Eccleston et al., 1965; Grahame-Smith, 1971b; Carlsson and Lindqvist, 1972; Glowinski and Axelrod, 1975; Mandel and Knapp, 1977; Fuller, 1980). PCPA inhibits irreversibly the brain tryptophan hydroxylase both in vivo and in vitro (Koe and Weissman, 1966; Jequier et al., 1967; Gal and Millard, 1971; Sanders-Bush and Massari, 1977). Above findings were supported by our results.

Several investigators have found that rats treated with compounds, which either dramatically increase synaptic serotonin or directly stimulate postsynaptic serotonin receptors, developed a stereo-

typed behavioral syndrome which consists most conspicuously of resting tremor, rigidity or hypertonus, reciprocal forepaw treading, Straub tail, hindlimb abduction, lateral head weaving, hyperreactivity and salivation(Corne et al., 1963; Grahame-Smith, 1971ab; Green and Youdim, 1975; Green and Grahame-Smith, 1976; Jacobs, 1976; Green and Kelly, 1976; Sloviter et al., 1978; Marsden and Curzon, 1978; Matthews and Smith, 1980; Ortman et al., 1980). A similar syndrome is also observed in amphetamine treated rats (Jacobs, 1976; Sloviter et al., 1978; Gerson and Baldessarini, 1980). A good deal of evidence indicated that above syndrome has specificity in reflecting the activity in the 5-HT system (Grahame-Smith, 1971[a,b]; Squires and Lassen, 1975; Jacobs, 1976; Crow and Deakin 1977; Deakin and Green, 1978; Sloviter et al., 1978; Gerson and Baldessarini, 1980).

However, there are two problems associated with the direct equation of these behavioral signs with central serotonergic stimulation. First, there is no good correlation between motor responses and neurochemical measurements of increased serotonergic function, such as levels of 5-HT, its synthesis or accumulation (Foldes and Costa, 1975). Second, the serotonin syndrome is significanly influenced by drugs that act primarily on dopaminergic neurotransmission(Jacobs et al., 1974; Green and Grahm-Smith, 1976; Green and Kelly, 1976).

In this experiment, at lower dose(2 mg/kg) d-amphetamine appeared more effective in causing hyperactivity, but at higher dose (6 mg/kg) d-amphetamine caused a greater incidence of stereotypy. And there was conversion from increase of locomotor activity to increase in stereotypy till 1 hr post injection in d-amphetamine treated rats. Only administration of L-tryptophan caused no gross behavioral changes because increased 5-HT turnover to 5-HIAA probably resulted in little 5-HT receptor activation. Pretreatment with L-tryptophan resulted in a more pronounced stimulation of stereotyped head weaving, forepaw treading and hindlimb abduction and a higher concentration of 5-HT but a less stimulation of locomotor activity and stereotyped gnawing and sniffing. But pretreatment with PCPA partially antagonized the stereotyped head weaving, forepaw treading and hindlimb abduction and the elevation of 5-HT which were induced by d-amphetamine but increased the locomotor activity and sterotyped gnawing and sniffing.

Our results strongly suggest that there is a strong positive correlation between the levels of 5-HT measured 1 hr after injection of d-amphetamine, PCPA plus d-amphetamine or L-tryptophan plus d-amphetamine and the numbers of occurrence of the stereotyped head weaving, forepaw treading and hindlimb abduction. In addition, a weak negative correlation between the levels of 5-HT and the numbers of occurrence of locomotor activity and of stereotyped gnawing and sniffing was found.

The present study suggests that 5-HT mechanism may play a stimulatory role in the expression of sterotyped head weaving, forepaw treading, and hindlimb abduction, but may probably exert inhibitory effect on the expression of locomotor activity, and stereotyped gnawing and sniffing.

In summary, It is concluded that changes of 5-HT turnover rate to 5-HIAA may be chiefly regulated by those of MAO activity evoked by d-amphetamine, and that 5-HT mechanism play a significant role in the expression of some of the behavioral syndrome induced by d-amphetamine.

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