J.Nerv.Ment.Dis. 124, 466 (1956).

EFFECTS OF PSYCHOTOMIMETIC COMPOUNDS ON CERTAIN OXIDATIVE AND HYDROLYTIC ENZYMES IN MAMMALIAN BRAIN[1,2]

LELAND C. CLARK, JR.,[3] R. PHYLLIS FOX,[3] RICHARD MORIN,[4] AND FREDERICK BENINGTON[4]

Among the ever-growing list of compounds which profoundly alter human behavior, mescaline remains of great interest partly because of the many structural analogs which may be synthesized. Analysis of the effects which mescaline and its isomers produce at a molecular, cellular, and psychic level (8) may provide insight as to the mechanism of action of the psychotomimetic drugs. As yet, the unique psychic effects of mescaline are not shared in a known way by a comparably unique effect on a cellular system. To date, the enzymatic approach has not provided information of value in understanding the activity of the psychotogens (1). Nonetheless, study of the relation between structure and enzymatic activity may suggest means by which structural antagonists may be prepared, means by which brain cells may be temporarily or permanently damaged, and perhaps someday, to help in understanding the relationship between mental disease and cellular metabolism. The work reported here is an extension of research (9) dealing with the direct effect of these compounds on enzyme systems *in vitro*.

Methods and Materials

A tissue preparation consisting of the cerebrum and underlying structures from the rat brain was used as the source of enzymes in this study. This tissue was homogenized with distilled water in an all-glass homogenizer followed by dilution appropriate for optimal measurement of the individual enzymes.

Partially purified alkaline phosphatase (AP), prepared by the method of Morton (13) was used in one phase of this study.

Alkaline phosphatase activity was measured in brain homogenates, supernatants from brain homogenates and partially purified brain alkaline phosphatase preparations. The method employed was that of Bessey, Lowry and Brock (7) as modified by Lowry *et al.* (12) for brain tissue, using p-nitrophenylphosphate as substrate and either glycine (.05 M) or 2-amino-2-methyl-1-propanol (AMP) (1.0 M) as buffer. The rate of tissue respiration utilizing pyruvate as substrate was measured by the method of Elliott *et al.* (10). For ease of discussion, the oxygen consumption measured this way is referred to in the text as "pyruvate utilization". Lactic and malic dehydrogenases (LDH and MDH) were determined by the spectrophotometric method of Strominger and Lowry (17).

Compounds to be tested were added to the various reaction mixtures by dissolving or suspending them in the buffer component, with pH readjustment where necessary. Special controls were run for compounds which produced color in the reaction mixture. In some instances, the insolubility or color development of a compound limited the concentration which could be used. All compound concentrations are expressed as the molarity existing in the reaction mixture during the period of incubation. Most of the primary β-phenethylamines and many of their analogs were prepared especially for use in this study (2-6). All the amines were used as hydrochlorides or in a few cases as the sulfate.

Results

Alkaline phosphatase and substituted phenethylamines

Typical pH curves for brain AP activity are shown in figure 1, illustrating the difference in activity attainable when two different buffers were used. Maximal activity in the glycine-buffer system was only 54 per cent of that found using 0.1 M AMP buffer. In a 1.0 M

[1] Read before the Eleventh Annual Meeting, Society of Biological Psychiatry, Chicago, April 28–29, 1956.

[2] This work was supported by a research grant (No. M-600) from the United States Public Health Service.

[3] Fels Research Institute, Antioch College, Yellow Springs, Ohio.

[4] Battelle Memorial Institute, Columbus, Ohio.

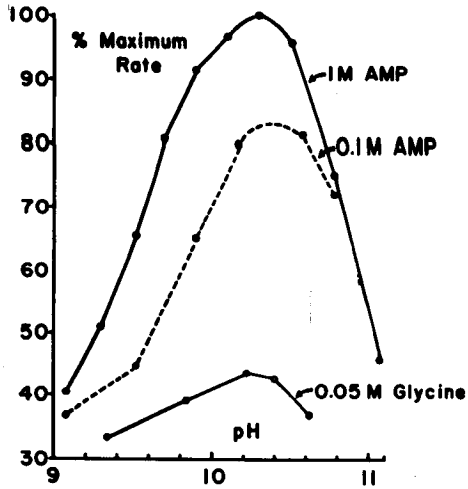


FIG. 1. A comparison of the relative effects of glycine and AMP buffers on brain alkaline phosphatase activity as influenced by pH. See text for details of enzyme reaction.

TABLE 1.—RELATION OF BRAIN ALKALINE PHOSPHATASE ACTIVITY TO CONCENTRATION OF 2-AMINO-2-METHYL-1-PROPANOL BUFFER

Buffer Concentration	Relative Enzyme Activity
(M)	
0.05	62.4
0.125	83.4
0.25	84.5
0.50	93.6
0.75	94.0
1.0	100.0

Reaction mixture: disodium p-nitrophenylphosphate (8 mM); $MgCl_2$ (2 mM); buffer, pH 10.4, as indicated above; brain homogenate to provide less than 4% hydrolysis of substrate during incubation at 37°C. for 30 minutes.

AMP buffer the enzyme activity was further increased. Similar pH curves were found for human serum AP activity. The quantitative relationship between AMP buffer concentration and AP activity is shown in table 1.

The fact that this enzyme shows very high activity in an amine buffer has special significance since it adds weight to the specificity of the inhibitions caused by the compounds being tested, most of which are cyclic amines present in a concentration $\frac{1}{100}$th that of the amine buffer used. It can be seen from table 2 that there was good agreement between the effects of the test compounds on AP activity in glycine and AMP buffer.

A series of 19 β-phenethylamines, including mescaline, was tested for effects on AP activity in order to study the relationship between structure and influence on enzyme activity. From this study (table 3) it is apparent that the *position* of substitution on the ring was more important than the *nature* of the substituent although the latter did exert some influence. In general, compounds which had substitutions in the 3,4,5 positions had only slight effects on the activity of the enzyme, whereas the unsubstituted compound, phenethylamine, exerted a definite inhibition.

Compounds having both the 2 and 6 positions alkyl- or alkoxy-substituted were more inhibitory to the enzyme than 3,4,5-substituted amines. Where the substitution was asymmetrical as in the 3-methoxy and the 2,3-methoxy compounds, there was also a fair degree of inhibitory action.

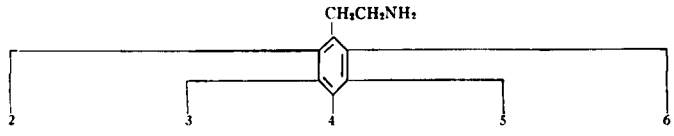
From an examination of 2,4,6-substituted compounds, it would appear that the degree of inhibitory effect was related to the nature

TABLE 2.—COMPARISON OF THE EFFECT OF COMPOUNDS ON BRAIN ALKALINE PHOSPHATASE ACTIVITY IN GLYCINE AND 2-AMINO-2-METHYL-1-PROPANOL (AMP) BUFFERS

Compound (10 mM)	Enzyme Activity (control = 100)					
	Glycine*			AMP†		
	N	Range	Mean	N	Range	Mean
Bufotenin	7	120–139	128	2	119–121	120
Serotonin	3	76–91	81	2	70–74	72
d-LSD-25	—	—	—	5	100–127	112
Mescaline	3	92–95	94	7	92–100	97

Reaction mixture as described in table 1.
* 0.05 M in reaction mixture.
† 1.0 M in reaction mixture.

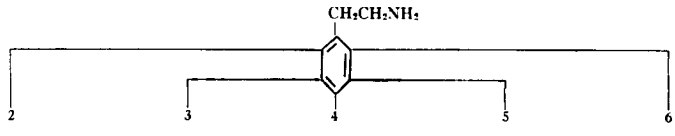
TABLE 3.—EFFECT OF PHENETHYLAMINES* ON BRAIN ALKALINE PHOSPHATASE ACTIVITY†



2	3	4	5	6	Activity (control = 100)
CH_3O	CH_3O	CH_3O	CH_3O	H	106
H	CH_3O	C_2H_5O	CH_3O	H	105
H	C_2H_5O	C_2H_5O	CH_3O	H	101
H	CH_3O	OH	CH_3O	H	97
H	CH_3O	CH_3O	CH_3O	H	97
H	CH_3O	CH_3O	H	H	90
CH_3O	CH_3O	CH_3O	H	CH_3O	90
H	H	H	H	H	89
H	CH_3O	OH	H	H	88
CH_3O	H	CH_3O	H	CH_3O	88
H	H	OH	H	H	87
CH_3O	CH_3O	H	CH_3O	CH_3O	86
CH_3	CH_3O	CH_3	CH_3	CH_3O	83
H	CH_3O	H	H	H	81
CH_3O	CH_3O	CH_3O	CH_3O	CH_3O	81
CH_3O	CH_3O	H	H	H	80
C_2H_5	H	C_2H_5	H	C_2H_5	77
C_2H_5O	H	C_2H_5O	H	C_2H_5O	57
CH_3	H	CH_3	H	CH_3	46

* 10 mM concentration of phenethylamines in reaction mixture.
† Reaction mixture as described in table 1 using 1.0 M AMP buffer.

TABLE 4.—EFFECT OF PHENETHYLAMINES* ON BRAIN RESPIRATION USING PYRUVATE SUBSTRATE†



2	3	4	5	6	Activity (control = 100)
H	CH_3O	OH	H	H	76
H	H	OH	H	H	76
H	H	H	H	H	72
CH_3O	CH_3O	H	H	H	69
H	CH_3O	H	H	H	63
H	CH_3O	OH	CH_3O	H	63
H	CH_3O	CH_3O	H	H	61
CH_3O	CH_3O	H	CH_3O	CH_3O	61
H	CH_3O	CH_3O	CH_3O	H	58
CH_3O	CH_3O	CH_3O	CH_3O	CH_3O	50
CH_3O	H	CH_3O	H	CH_3O	47
CH_3O	CH_3O	CH_3O	H	CH_3O	45
H	CH_3O	C_2H_5O	CH_3O	H	29
H	C_2H_5O	C_2H_5O	CH_3O	H	24
C_2H_5O	H	C_2H_5O	H	C_2H_5O	10
CH_3	CH_3O	CH_3	CH_3	CH_3O	9
CH_3	H	CH_3	H	CH_3	9
C_2H_5	H	C_2H_5	H	C_2H_5	5

* 10 mM concentration of phenethylamines in reaction mixture.
† Reaction mixture: 200 mg. brain; sodium pyruvate (.02 M); balanced salt solution to 3.0 ml.; pH 7.35, 37.5°C. for 1 hour.

TABLE 5.—EFFECT OF CERTAIN ANALOGS OF PHENETHYLAMINES ON BRAIN ALKALINE PHOSPHATASE ACTIVITY AND PYRUVATE UTILIZATION

Comp. No.	Compound*	Alkaline Phosphatase	Pyruvate Utilization
		(control = 100)	
1	benzylamine	78	81
2	phenethylamine	89	72
3	phenylisopropylamine (Dexedrine)	95	71
4	N,N-dimethylphenethylamine	106	88
5	N-methylphenylisopropylamine	100	71
6	N-methylcyclohexylisopropylamine (Benzedrex)	101	24
7	N-methylphenylisobutylamine (Wyamine)	117	61
8	benzoic acid	103	101
9	phenylacetic acid	103	95
10	benzamide	98	78
11	phenylacetamide	104	71
12	4-hydroxyphenethylamine	87	76
13	4-hydroxycyclohexylethylamine	86	53
14	4-methoxybenzylamine	66	75
15	4-methoxyphenylisopropylamine	74	61
16	3-amino-1-(4 methoxyphenyl)butane	63	27
17	N-benzyl-4-methoxyphenylisopropylamine	110	8
18	4-hydroxy-3-methoxyphenethylamine	88	76
19	4-hydroxy-3-methoxycyclohexylethylamine	96	70
20	3,4-dimethoxyphenethylamine	90	61
21	3,4-dimethoxyphenylisopropylamine	96	56
22	N-benzyl-3,4-dimethoxyphenylisopropylamine	97	11
23	N,N-dimethyl-3,4-dimethoxyphenethylamine	92	—
24	3,4-dimethoxyphenylacetic acid	107	102
25	3,4-dimethoxyphenylacetamide	104	78
26	3,4,5-trimethoxyphenethylamine	97	58
27	3,4,5-trimethoxyphenylisopropylamine	101	50
28	N-methyl-3,4,5-trimethoxyphenethylamine	105	64
29	N,N-dimethyl-3,4,5-trimethoxyphenethylamine	102	—
30	3,4,5-trimethoxybenzoic acid	104	74
31	3,4,5-trimethoxybenzamide	104	51
32	2,4,6-trimethoxyphenethylamine	88	47
33	2,4,6-trimethoxyphenylisopropylamine	90	28
34	2,4,6-trimethylphenethylamine	46	9
35	2,4,6-trimethylphenylacetic acid	101	72
36	2,4,6-trimethylphenylacetamide	114	49
37	N,N-diethyl-2,4,6-trimethylphenylacetamide	104	36

Reaction mixtures as described in tables 3 and 4.
* 10 mM concentration in reaction mixture.

of the substituent group as follows: $CH_3 > C_2H_5O > C_2H_5 > CH_3O$.

Pyruvate utilization and substituted phenethylamines

The same compounds had strikingly different effects on the "pyruvate utilization" system as shown in table 4. First, the *number* of substituents on the ring was a more significant factor in determining enzyme effect: in general, increasing the number of substitutions caused increased inhibition. And second, as can be seen from an examination of the 3,4,5-substituted compounds, the *nature* of the substituent

had a great influence. Replacing a hydroxy by a methoxy group increased inhibitory action, with still further inhibition brought about by ethoxy substitution. In the penta-substituted compounds, substitution of methyl groups for methoxy groups greatly increased inhibition.

The 2,4,6-substituted compounds were strong inhibitors of this enzyme system, as well as the AP system.

Mescaline analogs and enzyme activity

Table 5 shows the action of a series of side chain, N-substituted and ring analogs of mescaline on AP activity and pyruvate utilization. Certain generalizations can be made about the effect of such variations in structure on enzyme activity.

Increasing the *length of the side chain* as seen by examining compounds 1, 2, and 3; 14 and 15; 20 and 21; 26 and 27; 32 and 33 caused a lessening of inhibition of AP activity, but increased the inhibition of pyruvate utilization. *Saturation of the benzene ring* (seen by comparing compounds 5 and 6; 12 and 13; 18 and 19) had no significant effect on AP activity but did cause increased inhibition of the pyruvate system. *N-methyl substitution* (comparison of compounds 2 and 4; 3 and 5; 20 and 23; 26, 28 and 29) decreased the inhibitory effects of the amines on both enzyme systems, although in some cases only slightly. *N-benzyl substitution* in one case (comparing compounds 15 and 17) markedly decreased inhibition of AP activity, whereas no effect was observed in another case where the primary amine was not an inhibitor (comparing compounds 21 and 22). However, both these N-benzylated compounds strikingly increased the inhibition of the pyruvate system.

Several *amides* can be compared to their corresponding primary amines (compounds 1 and 10; 2 and 11; 20 and 25; 34 and 36). In the AP study, the amides were consistently less inhibitory than their corresponding amines, whereas the effects on the pyruvate system varied. In general the *carboxyl* derivatives were without effect on phosphatase but some depressed pyruvate utilization.

Other neuropharmacological agents and enzyme activity

A selection of psychotomimetic drugs, certain of their analogs, and several tranquilizers were studied for their effects on enzyme activity with results summarized in tables 6 and 7.

TABLE 6.—EFFECT OF PSYCHOTOMIMETIC COMPOUNDS, TRANQUILIZERS AND CERTAIN ANALOGS ON PREPARATIONS OF BRAIN ALKALINE PHOSPHATASE

Compounds (10 mM)	Enzyme Activity	
	Water homogenate	Purified preparation
	(control = 100)	
Mescaline	95	95
3,4,5-trimethoxyphenylisopropylamine	96	104
3,4-dimethoxyphenethylamine	84	89
Dexedrine	100	—
d-LSD-25	138	105
l-LSD-25	136	107
d-LAE-32	121	—
Serotonin	104	70
Bufotenin	154	112
Equanil	119	100
Reserpine	132	108
Chlorpromazine	130	103
Frenquel	140	110
Meratran	—	101

Reaction mixture as described in table 1, using glycine (.05 M) as buffer.

Two types of enzyme preparation were used in the AP study (table 6): one, a supernatant from a water homogenate of brain which had been allowed to settle for 3 hours in the cold; the other, a purified preparation of the enzyme referred to previously.

Using the first preparation of AP, mescaline and two of its analogs were mildly inhibitory. The lysergic acid derivatives, bufotenin, and the tranquilizers all showed activation of the enzyme with bufotenin exerting the strongest effect. Comparing these results with those obtained using the purified preparation of AP, it can be seen that the relative effect of these compounds was essentially the same, although the absolute values changed. Using the purified preparation, activation effects were reduced, and there was an increased inhibition due to serotonin.

A different pattern of effects appeared in the dehydrogenase studies (table 7). Mescaline and its analogs were slight stimulators of LDH. d-LSD-25 had no effect on this enzyme

TABLE 7.—EFFECT OF PYSCHOTOMIMETIC COMPOUNDS, TRANQUILIZERS AND CERTAIN ANALOGS ON LACTIC AND MALIC DEHYDROGENASE ACTIVITY

Compound	Lactic* Dehydrogenase		Malic† Dehydrogenase	
	Conc. of compound	Enzyme activity	Conc. of compound	Enzyme activity
	mM	control = 100	mM	control = 100
Mescaline	10	105	10	89
3,4,5-trimethoxyphenylisopropylamine	10	104	10	85
3,4-dimethoxyphenethylamine	10	104	10	97
Dexedrine	10	110	10	95
d-LSD-25	0.1	99	0.1	103
l-LSD-25	0.1	85	0.1	108
d-LAE-32	0.1	104	0.1	112
Serotonin	5	100	1	94
Bufotenin	5	81	1	94
Equanil	0.1	102	0.1	103
Reserpine	0.1	90	0.1	102
Chlorpromazine	0.1	102	0.1	93
Frenquel	0.1	98	0.1	99
Meratran	0.1	107	—	—

* Reaction mixture: sodium lactate (0.2 M); glycine (0.1 M); DPN (1 mM); pH 9.2; 12.5 c.mm. of 0.5% brain homogenate; 1.0 ml. volume; 25°C.

† Reaction mixture: potassium malate (0.2 M); glycine (0.1 M); DPN (2 mM); pH 10.2; 12.0 c.mm. of 0.25% brain homogenate; 1.0 ml. volume; 25°C.

while its psychotomimetically inactive l-isomer (11) caused inhibition. The serotonin-bufotenin relationship was the reverse of that seen with AP; bufotenin was an inhibitor of LDH while serotonin had no effect. Of the tranquilizers, reserpine exerted a slight inhibition while the others had practically no effect.

On the enzyme MDH, mescaline and its analogs had much the same inhibitory effect as on AP, with the most pronounced inhibition being shown by the isopropylamine analog. The lysergic acid derivatives were slight activators; serotonin and bufotenin had essentially no effect; and the tranquilizers, with the possible exception of chlorpromazine, showed no effect or a slight activation.

DISCUSSION

In the interpretation of data of the type presented here it is necessary to mention certain limitations in the method. The variability in activity of AP in tissue homogenates is well known and may be ascribed, at least partly, to the effects of naturally occurring inhibitors (15). It may be that the activation of this enzyme by tranquilizing agents is due to their action in overcoming such inhibitors. Less variability is observed in the dehydrogenase studies, due perhaps to the fact that a more dilute enzyme preparation is employed, materially diminishing the concentration of inhibitors. Specifically, the amounts of brain tissue per ml. of reaction mixture for each of the enzymes measured are as follows: AP, 2.6 mg.; LDH, 0.62 mg.; MDH, 0.29 mg.

It must be emphasized that relatively high concentrations of powerful drugs had to be used in certain instances to obtain significant effects on the enzymes studied. Such concentrations cannot be related to the *in vivo* state, however, since nothing is known about the concentration of a drug at enzyme sites in an intact cell.

Of the results reported in table 6, the activation effects of the tranquilizers as a group, and of bufotenin, are regarded as especially significant. Such activations are measurable in the presence of magnesium and AMP, both of which increase the activity of the enzyme. It has also been reported that AP activity is increased by phosphorylated vitamin D_2 (18), a finding which could not be confirmed with ester synthesized here.

The LDH enzyme system is relatively resistant to any of the compounds tested, suggesting that anaerobic cycles may be of little significance for this research. In contrast, the pyruvate utilization system was quite sensitive to the influence of the compounds tested, bearing out the findings of Quastel and Wheatley (14) and Schueler (16). It seems more likely that the psychotomimetic drugs exert their influence on aerobic systems and on phosphate metabolism.

The differential effects of the phenethylamines and their analogs on alkaline phosphatase and pyruvate utilization support the idea that an antagonist to mescaline might be found through enzyme studies of this type.

SUMMARY

In a study of mescaline and chemically related compounds, a series of nineteen phen-

ethylamines and thirty closely related analogs were found to exert strikingly differential effects on alkaline phosphatase activity and on pyruvate utilization in brain tissue preparations. In addition, ten other compounds of neuropharmacological interest were tested for their influence on alkaline phosphatase and on lactic and malic dehydrogenase. Bufotenin and the tranquilizers significantly increased phosphatase activity. The lactic and malic dehydrogenase systems were relatively resistant to the compounds tested.

The influence of the mescaline analogs on enzyme activity has been analyzed in terms of the structural configurations of these compounds.

The authors wish to acknowledge the generous supply of compounds provided as follows: bufotenin and serotonin creatinine sulfate from Dr. M. E. Speeter, The Upjohn Co.; d- and l-LSD-25 and LAE-32 from Dr. Kenneth Ericson, Sandoz Pharmaceuticals; 3,4-diethoxy-5-methoxyphenethylamine, 2,4,5-trimethyl-3,6-dimethoxyphenethylamine and 3-methoxyphenethylamine from Dr. P. Hey, Dept. of Pharmacology, University of Leeds, England; N-methyl-3,4,5-trimethoxyphenethylamine from Dr. L. A. Woods, Dept. of Pharmacology, University of Michigan Medical School; Dexedrine, Benzedrex, 4-methoxyphenylisopropylamine, 3-amino-1-(4-methoxyphenyl)butane, N-benzyl-4-methoxyphenylisopropylamine, 3,4-dimethoxyphenylisopropylamine, N-benzyl-3,4-dimethoxylphenylisopropylamine and chlorpromazine from Dr. Alfred R. Maass, Smith, Kline and French Laboratories; Wyamine and Equanil from Dr. W. F. Bruce, Wyeth Institute for Medical Research; reserpine from Dr. A. J. Plummer, Ciba Pharmaceutical Products; Frenquel and Meratran from Dr. Barbara Brown, The Wm. S. Merrill Company.

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