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THE ACTION OF LYSERGIC ACID DIETHYLAMIDE ON MAMMALIAN CHOLINESTERASES

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The psychological effects produced by administration of lysergic acid diethylamide (LSD) to man are now well known (see Stoll and Hofmann, 1943; Stoll, 1947, 1949; Condrau, 1949). Considerable information is also available about the general pharmacological effects in animals, but there is very little evidence bearing on the possible biochemical mode of action of this compound. Mayer-Gross, McAdam and Walker (1951, 1952) have described a slight transitory increase in the glucose and hexosemonophosphate levels in the blood following the administration of LSD, but carbohydrate metabolism did not otherwise appear to be affected; they suggested that the psychological symptoms may in part be due to an interruption of carbohydrate breakdown resulting from a block at the hexosemonophosphate stage. Liddell and Weil-Malherbe (1953), however, concluded that the effects of LSD on the blood sugar level were hardly significant, although they did describe changes in the blood adrenaline level.

Because of a similarity in molecular structure between lysergic acid and tryptamine, and because 5-hydroxytryptamine is known to be present in certain parts of the brain (Amin, Crawford and Gaddum, 1952), Gaddum (1953) carried out experiments to determine whether any interaction existed between these two drugs; he found that small amounts of LSD inhibit the action of 5-hydroxytryptamine on the rat's uterus.

Lewis and McIlwain (1954) have studied the action of LSD on the respiration and glycolysis of guinea-pig cerebral cortex; they found that when the tissue was stimulated by electrical pulses both these processes were inhibited by concentrations between 3×10^{-6}M and 10^{-4}M. Similar concentrations of both ergotoxine and dihydroergotamine were, however, also found to produce a similar degree of inhibition. They also reported that this effect of LSD was not prevented by the simultaneous presence of 5-hydroxytryptamine.

A further approach to the mode of action of this compound was initiated by Poloni and Maffezzoni (1952); these workers studied the levels of acetylcholine in the brains of guinea-pigs treated with bulbocapnine, mescaline and LSD; they found that LSD caused an increase in the acetylcholine level of the brain. Animals treated with bulbocapnine showed decreased amounts, whereas with mescaline the level was unchanged.

In view of this last report we have studied the action of LSD on some esterases, including the cholinesterases, of man and laboratory animals. Since LSD is a synthetic derivative of one of the constituents of ergot, a few experiments have also been carried out with ergotoxine, ergotamine, dihydroergotamine and ergometrine.

A preliminary report on this work was given to the Biochemical Society on July 16, 1954 (Thompson, Tickner and Webster, 1954).

METHODS

Estimation of Esterase Activity

Esterase activity was determined manometrically at 38° C. and at *p*H 7.4 using the Warburg technique (Ammon, 1933).

Substrates

(1) Acetylcholine perchlorate (ACh) (British Drug Houses, Ltd.).

(2) Butyrylcholine perchlorate (BuCh), prepared in this laboratory.

(3) Acetyl-β-methylcholine chloride (MCh) (Savory & Moore, Ltd.).

(4) Tributyrin (TB) (British Drug Houses, Ltd.).

The choline esters were dissolved in 0.025 M-$NaHCO_3$ solution immediately before use. The tributyrin was pipetted directly into the side-arm of the Warburg flasks (0.03 ml./flask), and was covered with 0.17 ml. of 0.025 M-$NaHCO_3$.

Materials

(1) Human serum.

(2) Human erythrocytes, washed once with saline and resuspended in distilled water, the final volume equalling the original volume of blood.

(3) Various areas were dissected from human brains obtained from cadavers. Because of the relatively high level of pseudo-cholinesterase activity in the white fibre tracts of human brain (Ord and Thompson, 1952, and later unpublished observations), the cerebral white matter (frontal lobe), internal capsule and corpus callosum were used as a source of this enzyme. The caudate nucleus was used for estimation of true cholinesterase.

(4) For experiments with rat, guinea-pig, rabbit, and chicken brain, the whole brain with the exception of the cerebellum was used. With monkey brain (*Macacus rhesus*) the internal capsule, corpus callosum and the white matter from the cerebrum were dissected and either pooled or the internal capsule used separately for pseudo-cholinesterase determinations. Cerebral cortex was used as a source of true cholinesterase.

In each experiment a weighed amount of the brain tissue was homogenized in 0.025 M-$NaHCO_3$. The homogenates, whose concentrations were chosen to give suitable CO_2 evolutions when measured over 0–60 min., were added to the main bulbs of the Warburg flasks.

Ergot Derivatives

(1) Lysergic acid diethylamide (Sandoz Products, Ltd.).

(2) Ergotoxine ethanesulphonate (British Drug Houses, Ltd.).

(3) Ergotamine tartrate (Sandoz Products, Ltd.).

(4) Dihydroergotamine methanesulphonate (Sandoz Products, Ltd.).

(5) Ergometrine maleate (Burroughs, Wellcome & Co.).

(6) Methylergometrine tartrate (Sandoz Products, Ltd.).

RESULTS

Effect of LSD on Esterases in Human Blood

The effect of LSD on the activity of pseudo-cholinesterase in human serum was first studied, using 0.015 M-ACh perchlorate as substrate. Under these conditions 10^{-6}M-LSD (=1 μg./ml.) caused about 50% inhibition of activity (Table I), whereas 2×10^{-5}M produced almost complete inhibition. Using 0.015 M-BuCh perchlorate as a selective substrate for pseudo-cholinesterase (Nachmansohn and Rothenberg, 1945), inhibition was again observed, although to a lesser extent than with ACh as substrate. On the other hand, the true cholinesterase in human erythrocytes was not affected by 2×10^{-5}M-LSD, and a concentration of 10^{-4}M produced only 19% inhibition.

The early observations of Vahlquist (1935), Easson and Stedman (1937) and Richter and Croft (1942) had suggested that the hydrolysis of aliphatic esters such as tributyrin by human plasma was brought about very largely by the plasma cholinesterase. The more recent work of Adams and Whittaker (1949) disclosed the presence in human plasma of a small amount of a second enzyme capable of hydrolysing TB and triolein, but these workers also concluded that in plasma the pseudo-cholinesterase is mainly responsible for TB hydrolysis. We therefore also used TB as substrate, the hydrolysis of which was found to be inhibited by low concentrations of LSD (Table I).

TABLE I

PERCENTAGE INHIBITION OF HUMAN ESTERASES BY LSD (1 μG. LSD/3 ML. = APPROX. 10^{-6}M)

Enzyme Source	Substrate	LSD Concn. (μg./3 ml.), and % Inhibition Produced						
		1	2	5	10	20	50	100
Plasma	ACh (0·015M)	49	66	87	89	98	—	—
,,	ACh (0·03M)	—	—	73	—	—	—	—
,,	ACh (0·06M)	—	—	58	—	—	—	—
,,	BuCh (0·015M)	24	40	63	74	84	—	—
,,	Tributyrin	70	79	89	91	94	—	—
Erythrocytes	ACh (0·015M)	—	—	3	0	0	16	19

TABLE II

PERCENTAGE INHIBITION FROM 5×10^{-6}M-LSD (=5 μG. 3 ML.) WITH VARYING SUBSTRATE CONCENTRATIONS

Enzyme Source	Substrate	Substrate Concn., and % Inhibition Produced					
		0·0019M	0·0037M	0·0075M	0·015M	0·03M	0·06M
Human serum	ACh	94	90	91	87	73	58
,, ,,	BuCh	88	90	71	63	36	19
,, erythrocytes ..	ACh	0	5	8	3	0	—

Table II shows the effect of variations in the concentration of ACh and BuCh on the inhibition produced by 5×10^{-6}M-LSD. With either substrate the degree of inhibition of the pseudo-cholinesterase diminishes as the substrate concentration increases; analysis of the data according to Lineweaver and Burk (1934) indicates that LSD inhibits competitively. The true cholinesterase in the red cells, on the other hand, remained insensitive to this concentration of LSD over the range of substrate concentrations studied.

Four experiments were done to discover whether the inhibition of cholinesterase by LSD is readily reversible or not. One volume of human serum was added (*a*) to 3 vols. of 0.025 M-$NaHCO_3$, and (*b*) to 3 vols. of 0.025 M-$NaHCO_3$ containing LSD. Samples of each were taken for estimation of the inhibition produced by the LSD (final concentration = 5 μg. LSD/3 ml.). After 15 min. at room temperature samples of (*a*) and (*b*) were diluted

5, 10 and 20-fold with 0.025 M-$NaHCO_3$, and the inhibition at each dilution estimated: in the undiluted flasks cholinesterase activity was 85% inhibited; after 5, 10 and 20-fold dilution the inhibition was 46, 24 and 18% respectively.

Effect of LSD on Brain Esterases

The pseudo-cholinesterase in human brain was next studied, using various white fibre tract areas

TABLE III
PERCENTAGE INHIBITION BY LSD OF HUMAN BRAIN ESTERASES

Enzyme and Source	Substrate	LSD Concn. (μg./3 ml.) and % Inhibition Produced			
		5	10	20	50
Pseudo-cholinesterase					
Frontal cerebrum (white)	ACh	66	60	63	60
,, ,, ,, ..	BuCh	54	69	83	88
Cerebellum	BuCh	—	44	—	—
Internal capsule ..	BuCh	—	76	—	—
Corpus callosum	BuCh	—	62	—	—
True cholinesterase					
Caudate nucleus ..	ACh	2	5	4	10
,, ,, ...	MCh	2	2	6	8
Cerebellum	MCh	—	0	—	—
Tributyrinase					
Frontal cerebrum (white)	TB	1	8	10	7
Caudate nucleus	TB	0	4	13	21

as well as the cerebellum as a source of enzyme and both ACh and BuCh as substrates. It will be seen from Table III that this enzyme in brain is also highly sensitive to inhibition by LSD. The true cholinesterase of human brain, on the other hand, using both caudate nucleus and the cerebellum as enzyme sources, appears to be almost completely insensitive to the concentrations of LSD listed in Table III, with either ACh or the selective substrate MCh (Mendel, Mundell, and Rudney, 1943) as substrate.

In contrast to the results obtained with human serum, TB hydrolysis by human brain appears to be very largely unaffected by these concentrations of LSD. This result agrees with earlier observations (Ord and Thompson, 1952) indicating that brain pseudo-cholinesterase does not contribute to any significant degree to the hydrolysis of TB.

TABLE IV
PERCENTAGE INHIBITION BY LSD OF BRAIN CHOLINESTERASE OF VARIOUS SPECIES

Species	Source of Enzyme	Substrate	LSD Concn. (μg./3 ml.) and % Inhibition Produced			
			5	10	20	50
Rat ..	Whole brain minus cerebellum	BuCh	9	3	5	6
		MCh	—	0	—	—
Guinea-pig	,, ,,	BuCh	2	4	13	3
		MCh	—	0	—	—
Rabbit	,, ,,	BuCh	12	11	23	37
Chicken	,, ,,	BuCh	3	4	7	0
Monkey	Internal capsule	ACh	6	—	—	—
	,,	BuCh	9	20	33	—
	Pooled white matter	BuCh	8	29	35	61
	Cerebrum (grey)	MCh	5	0	2	8

The effect of LSD on the cholinesterases in the brains of a number of animal species has also been studied. It will be seen (Table IV) that BuCh hydrolysis by rat, guinea-pig and chicken brain is hardly affected by concentrations of LSD which produce a very substantial inhibition in human brain. The pseudo-cholinesterase of rabbit brain seems slightly more sensitive, and that of monkey brain still more so, although their individual sensitivities are considerably less than that of human brain. True cholinesterase in rat, guinea-pig, and monkey brain appears to be unaffected by the concentrations used.

The results obtained with the other ergot alkaloids studied are shown in Table V. It will be seen that ergotoxine, ergotamine, and dihydroergotamine produce only slight inhibition (0–17%) of human serum cholinesterase at a concentration of 100 μg./3 ml. Ergometrine maleate, on the other hand, is a more potent inhibitor, though considerably less active than LSD; thus 5 μg.

TABLE V
PERCENTAGE INHIBITION OF HUMAN SERUM CHOLINESTERASE BY ERGOT DERIVATIVES, WITH ACh AS SUBSTRATE

Derivative	Concn. (μg./3 ml. of Derivative), and % Inhibition Produced						
	1	5	10	20	50	100	200
Ergotoxine ethanesulphonate	—	—	—	—	—	5	13
Ergotamine tartrate ..	—	—	—	—	—	17	22
Dihydroergotamine methanesulphonate	—	—	—	—	—	0	12
Ergometrine maleate	6	16	32	40	63	74	85
Methylergometrine tartrate	0	0	1	6	10	29	50

ergometrine maleate/3 ml. causes only 16% inhibition of serum cholinesterase, as compared with 87% produced by 5 μg. LSD/3 ml. Methylergometrine tartrate, although more active than ergometrine in its action on the pregnant rabbit's uterus, causes less inhibition of pseudo-cholinesterase.

In view of Gaddum's findings (1953) quoted earlier, we have done two experiments to determine whether the simultaneous presence of 5-hydroxytryptamine affects the inhibition of pseudo-cholinesterase by LSD; 10^{-4}M-5-hydroxytryptamine creatinine sulphate (kindly provided by Dr. E. Lester Smith) did not influence the inhibition caused by 2×10^{-6}M-LSD.

Discussion

The foregoing results indicate that LSD is a relatively powerful inhibitor of the pseudo-cholinesterase of human plasma, approximately 50% inhibition of enzymic activity being brought about by a concentration of 10^{-6}M. True cholinesterase in human erythrocytes is only slightly affected by concentrations that cause almost complete inhibition of pseudo-cholinesterase. The same relationship appears to hold for the cholinesterases in human brain, pseudo-cholinesterase being inhibited by concentrations of LSD which leave the true cholinesterase hardly affected. Table III shows that, when BuCh is used as substrate, increasing concentrations of LSD (up to 50 μg./3 ml.) cause increasing degrees of inhibition of brain pseudo-cholinesterase (up to 88%), whereas with ACh as substrate the inhibition remains at about 60% even though the concentration of LSD is increased tenfold up to levels which cause virtually complete inhibition of the plasma enzyme ; this is probably because with ACh as substrate both types of cholinesterase are estimated, so that even in the presence of amounts of LSD which cause complete inhibition of pseudo-cholinesterase there will still be a measurable activity owing to the LSD-insensitive true cholinesterase.

Tables I and III show that, whereas TB hydrolysis by human plasma is inhibited by low concentrations of LSD, the tributyrinase activity of brain is insensitive. As mentioned earlier, therefore, it would seem that the pseudo-cholinesterase of brain, unlike the plasma enzyme, does not hydrolyse TB.

The action of LSD differs in several respects from that of many of the more extensively studied inhibitors of cholinesterases ; thus eserine inhibits both true and pseudo-cholinesterases, whereas di*iso*propyl phosphorofluoridate (DFP), although showing some selective action towards pseudo-cholinesterase, will also inhibit true cholinesterase at slightly higher concentrations ; tri*ortho*cresyl phosphate inhibits *in vivo* both pseudo-cholinesterase and tributyrinase, although it is inactive against true cholinesterase. LSD appears to inhibit more specifically the pseudo-cholinesterase, leaving both true cholinesterase and tributyrinase in brain relatively unaffected. The seemingly rapid reversibility of the inhibitory action of LSD is another point of differentiation between this compound and the organo-phosphorus anti-cholinesterases.

The high sensitivity of human pseudo-cholinesterases to low concentrations of LSD, as compared with the pseudo-cholinesterases of the rat, guinea-pig, rabbit, chicken and even the monkey (Table IV) is of interest in connexion with the suggestions in the pharmacological and clinical literature that man is more sensitive to this compound than are most of the lower animals so far studied.

In general, our results with the other ergot alkaloids acting on human serum fall into line with those of earlier workers. Thus, Gautrelet and Scheiner (1939) claimed that ergotamine tartrate has only about 1/200th the anti-cholinesterase activity of eserine towards horse serum cholinesterase. Brügger (1938), using defibrinated cat's blood as a source of cholinesterase, concluded that both ergotamine and ergometrine were only weak inhibitors of cholinesterase. Navratil (1937), on the other hand, tested ergometrine on human serum, and obtained a degree of inhibition agreeing well with that found by us.

We cannot reach any conclusions from the work described here as to whether this action on pseudo-cholinesterase is relevant to the pharmacological effects produced by ingestion of this drug by man, although the finding of increased levels of ACh in the brains of guinea-pigs treated with LSD (Poloni and Maffezzoni, 1952) would be consistent with the presence of cholinesterase inhibition *in vivo*.

Mental changes following the administration of another cholinesterase inhibitor, di*iso*propyl phosphorofluoridate, have also been described, although it does not necessarily follow that the changes were directly due to the anti-cholinesterase action of the DFP in the brain. For example, Rowntree, Nevin, and Wilson (1950) reported that the daily administration of DFP to normal subjects and to depressive patients caused depressant effects, whereas, when administered to a number of schizophrenic patients, an activation of the psychosis was observed. Aprison, Nathan, and Himwich (1954) have also described abnormal behaviour patterns in rabbits, involving circling and compulsive turning movements, associated with inhibition of brain cholinesterase activity by injection of DFP into the common carotid artery. It must be pointed out, however, that recent evidence strongly suggests that pseudo-cholinesterase in the nervous system is a component not of the neurones but of the glial and Schwann cells (Cavanagh, Thompson, and Webster, 1954). Indeed, since the physiological significance of this enzyme in the central nervous system is at present largely unknown, the findings reported here must be interpreted with caution.

SUMMARY

1. The action of lysergic acid diethylamide (LSD) on the cholinesterases of man and a number of other animal species is described.

2. LSD is a relatively powerful inhibitor of pseudo-cholinesterase in human serum and brain.

3. The true cholinesterase and tributyrinase of human brain are only slightly affected by concentrations of LSD that cause an almost complete inhibition of pseudo-cholinesterase.

4. Inhibition of pseudo-cholinesterase by LSD is competitive in nature, and is readily reversible.

5. Pseudo-cholinesterase activity of rat, guinea-pig, rabbit, chicken and monkey brain is much less sensitive to inhibition by LSD than is the corresponding enzyme in human brain.

6. LSD is a more powerful inhibitor of pseudo-cholinesterase than are the other ergot alkaloids studied.

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