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SHORT COMMUNICATIONS

Studies on the *in vitro* metabolism of compounds related to lysergic acid diethylamide (LSD)

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IN A PREVIOUS paper[1] it was shown that D-lysergic acid diethylamide (LSD) was transformed to D-lysergic acid monoethylamide (LAE) and D-N^6-demethyl-lysergic acid diethylamide (norLSD) by an enzyme in liver microsomes supplemented with NADPH and oxygen.

Many LSD-related compounds are now available which show a variety of physiological actions. In most of these compounds, the side chain at position 8 of lysergic acid, the hydrogen at position 1 or the hydrogen at position 2 are substituted by an alkyl or alcohol amide group, a methyl group, or a bromine group, respectively. In order to find some clues to the mechanism of the hallucinogenic action of LSD, the metabolic pathways of these derivatives were investigated. The present paper described the metabolism of several lysergic acid derivatives by rat liver 9000 *g* supernatant.

The substrates used in these experiments were as follows: D-2-bromolysergic acid diethylamide (BOL) which has a strong antagonistic action to 5-hydroxytryptamine (5HT) in peripheral organs[2] but no effect on the central nervous system[3]; 1-methyl-D-lysergic acid (+)-butanolamide-(2) (methysergide) which has an antagonistic action to 5HT in both the peripheral[4] and central organs[5] but no hallucinogenic action; D-lysergic acid (+)-butanolamide-(2)(methergine) which has an antagonistic action to 5HT in peripheral organs but no action in the central nervous system[2]; D-lysergic acid dimethylamide (LDM) and D-lysergic acid amide (LAA) which have hallucinogenic actions about one tenth that of LSD[6]; and isomers such as *iso*LSD, *iso*LDM, *iso*methergine and *iso*LAA.

LSD, LDM, LAA, and methergine were prepared from D-lysergic acid by Garbrecht's method,[7] their isomers being obtained as by-products, and BOL was prepared from LSD by the method of Troxler *et al.*[8] Methysergide was supplied by Sandoz Ltd., Basel, Switzerland. NADP and NADPH were obtained from Sigma Chemical Co., Ltd., St. Louis, U.S.A. SKF 525-A was kindly supplied by Professor Kitagawa, University of Chiba, Japan. All other chemicals were special grade and purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

The 9000 *g* supernatant and microsomal fractions were prepared from the livers of male Wistar rats weighing 150–200 g as described previously.[1] The incubation mixture consisted of 3 ml of 9000 *g* supernatant fraction, 0·5–3·0 μmoles of substrate, 1 μmole of NADP, 100 μmoles of nicotinamide, 100 μmoles of $MgCl_2$, 400 μmoles of phosphate buffer (pH 7·4), and water to a final volume of 5 ml. The system was incubated for 2 hr, at 37°, with shaking. Unchanged substrate and metabolites were extracted and determined as described previously.[1] As shown in Table 1, in the complete system maximal enzyme activity was obtained with each substrate and it was concluded that the substrates were being metabolized by an enzyme in rat liver microsomes, except LAA and *iso*LAA which were transformed to *iso*LAA and LAA, respectively, both under anaerobic conditions and with heated extract. LAA and *iso*LAA were thus isomerized non-enzymically during incubation. Since 10^{-3} M SKF 525-A blocked the metabolism of the substrates this supported the view that the transformations were enzymatic. Metabolites were isolated and detected on thin-layer chromatography (t.l.c.). On thin-layer chromatograms of extracts of incubation mixtures with the complete system, the following spots, which gave a blue fluorescence under a u.v. lamp and/or a fast reaction with Van Urk's reagent, were observed apart from spots for the unchanged substrate: spot, A_1, for *iso*LSD; three spots, B_1, B_2 and B_3 which gave only the blue fluorescence but no colour with the reagent, for BOL; two spots, C_1 and C_2, for LDM; a spot, D_1, for *iso*LDM; a spot, E_1, for LAA; a spot, F_1, for *iso*LAA; two spots, G_1 and G_2, for methysergide; two spots, H_1 and H_2 for methergine, and one spot, I_1, for *iso*methergine (Table 2).

Metabolites present in $CHCl_3$ extracts of large scale incubation mixtures were purified by repeating preparative t.l.c. The data obtained by u.v. spectrometry and high resolution mass spectrometry of the metabolites are shown in Table 3.

The structures of the metabolites were determined by shift technique analysis of the ion fragments in the mass spectra of lysergic acid derivatives.[9] The position of hydroxylation in metabolite, H_2, has not yet been clarified.

TABLE 1. THE REQUIREMENTS AND THE INHIBITION OF THE METABOLISM OF COMPOUNDS RELATED TO LSD

Substrate	Substrate metabolized (%) 9000 *g* supernatant with NADP (complete system)	Complete system under anaerobic condition	Complete system with heated 9000 *g* supernatant	Microsomes with NADPH	Microsomes	Complete system with SKF 525-A (1×10^{-3} M)
*iso*LSD	53·1	5·2	6·0	43·2	5·0	8·2
BOL	62·8	0	0·4	51·0	0	1·7
LDM	48·4	0	0	38·5	0	2·5
*iso*LDM	45·1	2·4	1·1	36·0	2·5	2·6
LAA	49·7	21·8	22·1	44·7	21·9	22·5
*iso*LAA	83·5	49·7	45·9	77·8	46·0	49·9
Methysergide	63·4	0	0	35·2	0	0
Methergine	27·3	0	3·1	21·6	1·0	3·7
*iso*Methergine	80·7	0	0	65·0	0	2·3

TABLE 2. R_f VALUES OF METABOLITES OF LSD-RELATED COMPOUNDS ON THIN-LAYER CHROMATOGRAMS

Substrate and metabolite	Developing solvent and R_f value			Substrate and metabolite	Developing solvent and R_f value		
	(I)	(II)	(III)		(I)	(II)	(VII)
*iso*LSD	0·46	0·26	0·49	LAA	0·27	0·20	0·45
A_1	0·64	0·18	0·30	E_1	0·13	0·07	0·29
	(IV)	(V)	(VI)		(I)	(II)	(VII)
BOL	0·60	0·56	0·36	*iso*LAA	0·55	0·58	0·29
B_1	0·45	0·38	0·17	F_1	0·20	0·14	0·39
B_2	0·34	0·28	0·29		(I)	(II)	(X)
B_3	0·21	0·11	0·07	Methysergide	0·73	0·55	0·51
	(I)	(II)	(VII)	G_1	0·55	0·42	0·28
LDM	0·59	0·61	0·49	G_2	0·43	0·24	0·16
C_1	0·51	0·72	0·33		(I)	(II)	(X)
C_2	0·34	0·27	0·16	Methergine	0·55	0·42	0·28
	(I)	(VIII)	(IX)	H_1	0·32	0·23	0·14
*iso*LDM	0·19	0·61	0·39	H_2	0·24	0·14	0·08
D_1	0·27	0·51	0·52		(I)	(II)	(IV)
				*iso*Methergine	0·67	0·52	0·55
				I_1	0·23	0·16	0·04

Numeral in parenthesis represents solvent system for development in t.l.c. (I): MeOH–$CHCl_3$ (1:4, v/v), (II): acetone–AcOEt dimethylformamide (5:5:1, v/v), (III): diethylamine–$CHCl_3$ (1:9, v/v), (IV): acetone–$CHCl_3$ (4:1, v/v), (V): acetone–$CHCl_3$ (1:1, v/v), (VI); cyclohexane–$CHCl_3$–diethylamine (5:4:1, v/v), (VII): MeOH–$CHCl_3$ (4:1, v/v), (VIII): MeOH–diethylamine–$CHCl_3$ (0·2:1:9, v/v), (IX): MeOH–$CHCl_3$ (1:3, v/v), (X): MeOH–$CHCl_3$–*n*-hexane (1:4:2, v/v).

TABLE 3. METABOLITES OF LSD-RELATED COMPOUNDS

Spot on t.l.c.	u.v. $\lambda_{max\,nm}^{EtOH}$	Mass measurement for M^+ Found	Cald.	Formula	Metabolites found
A_1	312	309·186	309·184	$C_{19}H_{23}N_3O$	nor-*iso*LSD
B_1	303	373·078 375·076	373·079 375·077	$C_{18}H_{20}N_3OBr$	2-bromoLAE
B_2	303	387·092 389·092	387·094 389·092	$C_{19}H_{22}N_3OBr$	norBOL
B_3	303	359·063 361·061	359·063 361·061	$C_{17}H_{18}N_3OBr$	2-bromo-norLAE
C_1	312	281·153	281·153	$C_{17}H_{19}N_3O$	LAM*
C_2	312	281·152	281·153	$C_{17}H_{19}N_3O$	norLDM
D_1	312	281·152	281·153	$C_{17}H_{19}N_3O$	nor-*iso*LDM
E_1	312	253·121	253·122	$C_{15}H_{15}N_3O$	norLAA
F_1	312	253·121	253·122	$C_{15}H_{15}N_3O$	nor-*iso*LAA
G_1	312	339·196	339·195	$C_{20}H_{25}N_3O_2$	methergine
G_2	312	369·203	369·205	$C_{21}H_{27}N_3O_3$	N^1-hydroxymethyl methergine
H_1	312	325·179	325·179	$C_{19}H_{23}N_3O_2$	nor-methergine
H_2	312	355·190	355·190	$C_{20}H_{25}N_3O_3$	lysergic acid butanolamide
I_1	312	325·180	325·179	$C_{19}H_{23}N_3O_2$	nor-*iso*methergine

* LAM: lysergic acid monomethylamide

From the above results, it was found that the enzymatic demethylation of lysergic acid derivatives at position 6 to form nor-derivatives was independent of their physiological actions, and that a side chain at position 8 of normal lysergic acid derivatives was enzymatically attacked to form monoalkylamides or hydroxyalkyl amides. However, an exception occurs when the compound possesses a methyl group at position 1 for then demethylation occurs at position 1 instead of 6.

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Effect of 2-piperazino-4(3H)-quinazolinone monoacetate on the tissue respiration, glucose uptake and lactic acid production by rat hemidiaphragm*

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2-PIPERAZINO-4(3H)-quinazolinone monoacetate (I), is a hypoglycemic agent in some species of normal animals.[1] Also it can effectively lower blood sugar in different species of diabetic animals.[2] When administered by gastric intubation, it causes a remarkable increase in lactic acid and a lowering of liver and muscle glycogen in albino rats.[3] Its effect *in vitro* on the glucose uptake, lactic acid formation and tissue respiration of rat hemidiaphragm suspended in phosphate buffer has been studied and the results are reported.

Albino rats of the Charles Foster strain, body wt 110–160 g, were used. After fasting for 18 hr the rats, with constant access to water, were quickly decapitated and diaphragms were dissected out and collected in phosphate buffer at 4°.[4] Oxygen was bubbled through the buffer for 5 min at room temperature and then for 1 min after the hemidiaphragms had been transferred to Warburg flasks and suspended in 2 ml buffer containing 3 mg glucose/ml, cooled to 4°. (I) was added to give a final concentration of 0, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M. The flasks were incubated at 37° for 90 min with constant shaking. Manometric readings were taken at 30, 60 and 90 min. Glucose and lactic acid were estimated according to Sunderman *et al.*[5] and Barker and Summerson,[6] respectively, after 90 min incubation. The hemidiaphragms were weighed on a torsion balance after carefully pressing between two filter papers to remove the buffer solution. The weight of hemidiaphragms ranged from 120 to 200 mg.

There was an increase in glucose uptake and lactic acid production by diaphragms in flasks containing 10^{-4} M and 10^{-5} M(I) compared with the untreated control flasks (Table 1). There was no significant change in glucose uptake or lactic acid production in the presence of 10^{-3} M or 10^{-6} M(I).

It is evident from Fig. 1 that 10^{-4} M(I) caused maximum O_2 uptake by hemidiaphragm. On the other hand 10^{-3} M(I) inhibited the tissue respiration and the stimulating effects of (I) gradually waned beyond 10^{-4} M.

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