

SHORT COMMUNICATIONS

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

(Received 8 March 1974; accepted 6 April 1974)

IN A PREVIOUS paper¹ it was shown that D-lysergic acid diethylamide (LSD) was transformed to D-lysergic acid monoethylamide (LAE) and D-N⁶-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 organs² but no effect on the central nervous system³; 1-methyl-D-lysergic acid (+)-butanolamide-(2) (methysergide) which has an antagonistic action to 5HT in both the peripheral⁴ and central organs⁵ 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 system²; D-lysergic acid dimethylamide (LDM) and D-lysergic acid amide (LAA) which have hallucinogenic actions about one tenth that of LSD⁶; and isomers such as *iso*LSD, *iso*LDM, *isomethergine* 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 μ moles of substrate, 1 μ mole of NADP, 100 μ moles of nicotinamide, 100 μ moles of MgCl₂, 400 μ moles 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, A₁, for *iso*LSD; three spots, B₁, B₂ and B₃ which gave only the blue fluorescence but no colour with the reagent, for BOL; two spots, C₁ and C₂, for LDM; a spot, D₁, for *iso*LDM; a spot, E₁, for LAA; a spot, F₁, for *iso*LAA; two spots, G₁ and G₂, for methysergide; two spots, H₁ and H₂ for methergine, and one spot, I₁, for *isomethergine* (Table 2).

Metabolites present in CHCl₃ 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, H₂, has not yet been clarified.

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

Substrate	9000 g supernatant with NADP (complete system)	Complete system under anaerobic condition	Substrate metabolized (%)		Complete system with SKF 525-A (1×10^{-3} M)
			Complete system with heated 9000 g supernatant	Microsomes with NADPH	
isoLSD	53.1	5.2	6.0	43.2	8.2
BOL	62.8	0	0.4	51.0	1.7
LDM	48.4	0	0	38.5	2.5
isoLDM	45.1	2.4	1.1	36.0	2.6
LAA	49.7	21.8	22.1	44.7	22.5
isoLAA	83.5	49.7	45.9	77.8	49.9
Methysergide	63.4	0	0	35.2	0
Methergine	27.3	0	3.1	21.6	3.7
isoMethergine	80.7	0	0	65.0	2.3

TABLE 2. R_f VALUES

Substrate and metabolite
isoLSD A ₁
BOL B ₁ B ₂ B ₃
LDM C ₁ C ₂
isoLDM D ₁

Numeral in
MeOH-CHCl₃
diethylamine-
(1:1, v/v), (VI)
(4:1, v/v), (VII)
(1:3, v/v), (X)

Spot on t.l.c.	U.V. $\lambda_{\text{max nm}}$
A ₁	312
B ₁	303
B ₂	303
B ₃	303
C ₁	312
C ₂	312
D ₁	312
E ₁	312
F ₁	312
G ₁	312
G ₂	312
H ₁	312
H ₂	312
I ₁	312

* LAM: lysergic acid

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)
isoLSD	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
BOL	(IV)	(V)	(VI)	isoLAA	(I)	(II)	(VII)
B ₁	0.60	0.56	0.36	F ₁	0.55	0.58	0.29
B ₂	0.45	0.38	0.17		0.20	0.14	0.39
B ₃	0.34	0.28	0.29	Methysergide	(I)	(II)	(X)
	0.21	0.11	0.07	G ₁	0.73	0.55	0.51
LDM	(I)	(II)	(VII)	G ₂	0.55	0.42	0.28
C ₁	0.59	0.61	0.49		0.43	0.24	0.16
C ₂	0.51	0.72	0.33	Methergine	(I)	(II)	(X)
	0.34	0.27	0.16	H ₁	0.55	0.42	0.28
isoLDM	(I)	(VIII)	(IX)	H ₂	0.32	0.23	0.14
D ₁	0.19	0.61	0.39		0.24	0.14	0.08
	0.27	0.51	0.52	isoMethergine	(I)	(II)	(IV)
				I ₁	0.67	0.52	0.55
					0.23	0.16	0.04

Numerals in parenthesis represents solvent system for development in t.l.c. (I): MeOH-CHCl₃ (1:4, v/v), (II): acetone-AcOEt-dimethylformamide (5:5:1, v/v), (III): diethylamine-CHCl₃ (1:9, v/v), (IV): acetone-CHCl₃ (4:1, v/v), (V): acetone-CHCl₃ (1:1, v/v), (VI): cyclohexane-CHCl₃-diethylamine (5:4:1, v/v), (VII): MeOH-CHCl₃ (4:1, v/v), (VIII): MeOH-diethylamine-CHCl₃ (0.2:1:9, v/v), (IX): MeOH-CHCl₃ (1:3, v/v), (X): MeOH-CHCl₃-n-hexane (1:4:2, v/v).

TABLE 3. METABOLITES OF LSD-RELATED COMPOUNDS

Spot on t.l.c.	u.v. $\lambda_{\text{max}}^{\text{EtOH}}$	Mass measurement for M ⁺			Metabolites found
		Found	Cald.	Formula	
A ₁	312	309.186	309.184	C ₁₉ H ₂₃ N ₃ O	nor-isoLSD
B ₁	303	373.078	373.079	C ₁₈ H ₂₀ N ₃ OBr	2-bromoLAE
		375.076	375.077		
B ₂	303	387.092	387.094	C ₁₉ H ₂₂ N ₃ OBr	norBOL
		389.092	389.092		
B ₃	303	359.063	359.063	C ₁₇ H ₁₈ N ₃ OBr	2-bromo-norLAE
		361.061	361.061		
C ₁	312	281.153	281.153	C ₁₇ H ₁₉ N ₃ O	LAM*
C ₂	312	281.152	281.153	C ₁₇ H ₁₉ N ₃ O	norLDM
D ₁	312	281.152	281.153	C ₁₇ H ₁₉ N ₃ O	nor-isoLDM
E ₁	312	253.121	253.122	C ₁₅ H ₁₅ N ₃ O	norLAA
F ₁	312	253.121	253.122	C ₁₅ H ₁₅ N ₃ O	nor-isoLAA
G ₁	312	339.196	339.195	C ₂₀ H ₂₅ N ₃ O ₂	methergine
G ₂	312	369.203	369.205	C ₂₁ H ₂₇ N ₃ O ₃	N ¹ -hydroxymethyl methergine
H ₁	312	325.179	325.179	C ₁₉ H ₂₃ N ₃ O ₂	nor-methergine
H ₂	312	355.190	355.190	C ₂₀ H ₂₅ N ₃ O ₃	lysergic acid butanolamide
I ₁	312	325.180	325.179	C ₁₉ H ₂₃ N ₃ O ₂	nor-isomethergine

* 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.

First Chemistry Section,
National Research Institute of
Police Science,
Chiyoda-ku, Tokyo, Japan

TETSUKICHI NIWAGUCHI
TAKAKO INOUE
TOKIYASU SAKAI

REFERENCES

1. T. NIWAGUCHI, T. INOUE and Y. NAKAHARA, *Biochem. Pharmac.* 23, 1073 (1974).
2. E. ROTHLIN, *Ann. N.Y. Acad. Sci.* 66, 668 (1957).
3. N. KAWAI and C. YAMAMOTO, *Int. J. Neuropharmac.* 8, 437 (1969).
4. G. BALDRATTI, G. ARCARI and G. K. SUCHOWSKY, *Experientia* 21, 396 (1965).
5. W. P. KOELLA and J. CZICMAN, *Am. J. Physiol.* 211, 926 (1966).
6. A. HOFFER and H. OSMOND, in *The Hallucinogens*, p. 94. Academic Press, New York (1967).
7. W. L. GARBRECHT, *J. org. Chem.* 24, 368 (1959).
8. F. TROXLER and A. HOFMANN, *Helv. Chim. Acta* 40, 2160 (1957).
9. T. INOUE, Y. NAKAHARA and T. NIWAGUCHI, *Chem. Pharm. Bull., Tokyo* 20, 409 (1972).

Biochemical Pharmacology, Vol. 23, pp. 3066-3067. Pergamon Press, 1974. Printed in Great Britain.

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

(Received 10 December 1973; accepted 19 March 1974)

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°. 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 O₂ 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.

* Communication No. 1915 from Central Drug Research Institute, Lucknow, India.

TABLE 1. SHOWING GLUCOSE

Glucose uptake (µg glucose/ml buffer/mg diaph.)
Lactic acid (µg lactic acid/ml buffer/mg diaph.)
Oxygen uptake (µl O ₂ uptake/ mg diaph.)

Results expressed as mean

50
40
30
20
10
% O₂ uptake

Min

FIG. 1. Percentage oxygen u

An optimal concentration
by hemidiaphragms *in vitro*.
The *in vitro* effect shown
has been envisaged for the

Acknowledgements—The au
(I). Sincere thanks are also d
cal assistance.

Division of Toxicology and
Experimental Medicine.
Central Drug Research Inst
Post Box No. 173.
Lucknow-226001, India

1. C. M. GUPTA, S. T. H. 223, 524 (1969).
2. S. K. MUKHERJEE, *Bio*
3. S. K. MUKHERJEE and
4. D. F. STEINER and R. I
5. F. W. SUNDERMAN, JR.
6. S. B. BARKER and W. I
7. J. STERNE, in *Oral Hyp*
G. D. CAMPBELL) Vol.