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EFFECT OF LYSERGIC ACID DERIVATIVES ON THE LIVER FLUKE *FASCIOLA HEPATICA**

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Summary—Lysergic acid diethylamide derivatives, and a number of indole compounds related to serotonin, have been tested on preparations from the liver fluke *Fasciola hepatica* attached to a force transducer, and the concentrations which stimulated 50 per cent of these preparations (ED_{50}) have been determined. These derivatives can be arranged in an ascending order according to their ED_{50} as follows: D-lysergic acid diethylamide, D-lysergic acid pyrrolidide, D-lysergic acid dimethylamide, methyl-D-lysergic acid diethylamide, D-lysergic acid monoethylamide, D-lysergic acid morpholide, 1-methyl-D-lysergic acid butanolamide, bromo-D-lysergic acid diethylamide, and L-lysergic acid diethylamide. All these compounds except for bromo-D-lysergic acid diethylamide and L-lysergic acid diethylamide had a lower ED_{50} than serotonin and its related derivatives. There is a good correlation between the stimulant potency of these agents on the liver fluke and the reported hallucinatory effects on man.

INTRODUCTION

LYSERGIC acid diethylamide (LSD) and 5-hydroxytryptamine (serotonin) were shown to increase the rhythmical motility of the liver fluke *Fasciola hepatica* (MANSOUR, 1957). The structure–activity relation of LSD derivatives and other indoleamines have been investigated in man (ISBELL *et al.*, 1959) rat uterus (CERLETTI and DOEPFNER, 1958) and clam heart (WRIGHT *et al.*, 1962).

In the present investigation a number of LSD derivatives were tested on the liver fluke rhythmical motility and their effects were compared. The relationship between results obtained on the liver fluke preparations and those reported with other tissues will be discussed.

METHODS

Liver flukes were collected from infected cattle and suspended in a 15 ml organ bath as has been described before (CHANCE and MANSOUR, 1949; MANSOUR, 1957). The flukes were attached to a Grass force transducer which was calibrated to apply a tension equivalent to a half gram weight. Signals were recorded on a Grass polygraph.

Separate flukes were used for each tested concentration of a drug. A simple plus-minus response criterion was used to describe whether the drug stimulated or not. A stimulated

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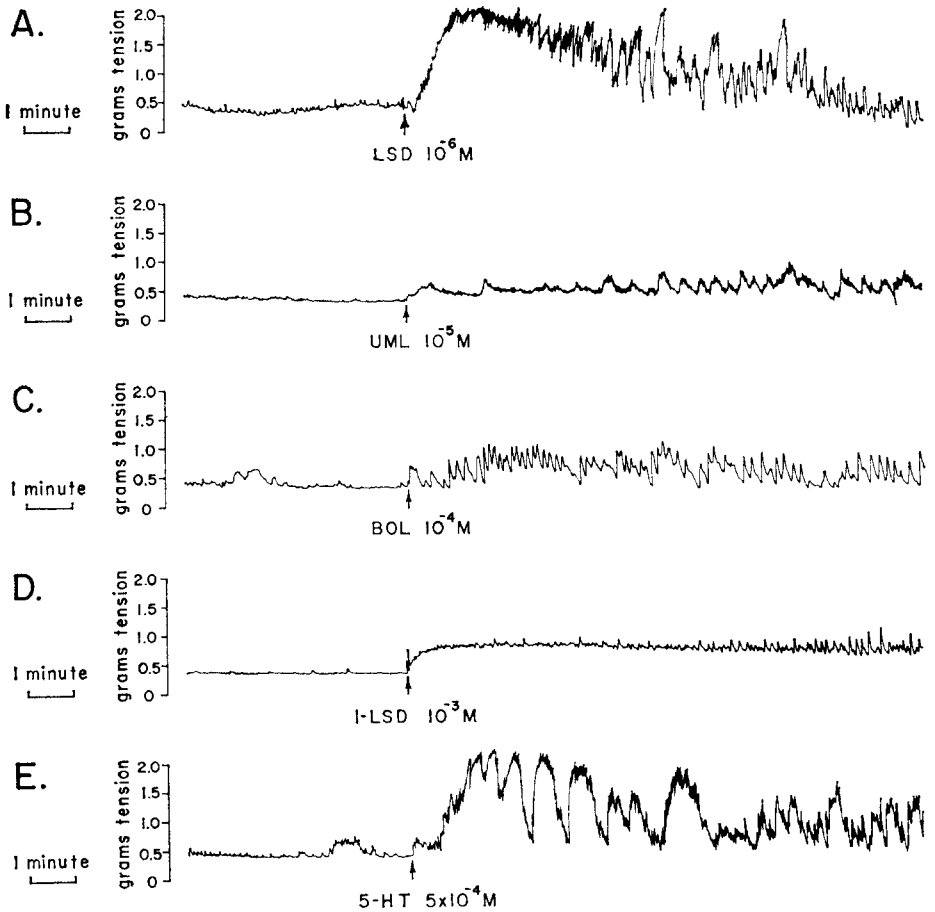


FIG. 1. Representative tracings of the fluke preparations showing the action of lysergic acid diethylamide and related compounds. Abbreviations for compounds are explained in Table 1. Drugs were added at the points indicated by the arrows. The standard tension calibration for the Grass force transducer is shown on the left side of every record.

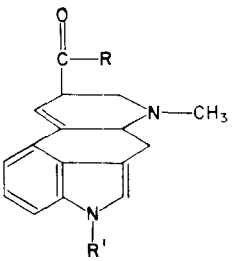
preparation was characterized by an increase in frequency or amplitude of contraction with or without change in tone (Fig. 1). The percentage of fluke preparations which were stimulated at a certain drug concentration was determined. For the purpose of obtaining quantitative data descriptive of the potencies of these compounds the effective dose which caused stimulation of half the total number of the fluke preparations (ED_{50}) was calculated according to the logit transformation of Berkson (1953). The chemical structure and abbreviations of the different lysergic acid derivatives are listed in Table 1.

RESULTS

Stimulant effect of lysergic acid derivatives

Of all the compounds tested, LSD was the most potent compound. It caused immediate stimulation of 100 per cent of the fluke preparations at a concentration of 10^{-6}M and had an ED_{50} of $4{\cdot}8 \times 10^{-8}$M (Table 2). The stimulant effect was characterized by an immediate

TABLE 1. CHEMICAL STRUCTURE AND ABBREVIATIONS OF D-LYSERGIC ACID DERIVATIVES TESTED.



Compounds	R	R^1	Abbreviations
D-Lysergic acid diethylamide	$-CO-N(C_2H_5)_2$	H	LSD
D-Lysergic acid pyrrolidide	$-CO-N(CH_2-CH_2)_2$	H	LPD
D-Lysergic acid dimethylamide	$-CO-N(CH_3)_2$	H	DAM
N-Methyl-D-lysergic acid diethylamide	$-CO-N(C_2H_5)_2$	CH_3	MLD
D-Lysergic acid monoethylamide	$-CO-NH-C_2H_5$	H	LAE
D-Lysergic acid morpholide	$-CO-N(CH_2-CH_2)_2O$	H	LSM
1-Methyl-D-lysergic acid butanolamide (methysergide)	$-CO-NH-C_4H_8OH$	CH_3	UML
2-Bromo-D-lysergic acid diethylamide*	$-CO-N(C_2H_5)_2$	H	BOL
L-Lysergic acid diethylamide	$-CO-N(CH_2H_5)-C_2H_5$	H	L-LSD

* A bromine atom is substituted in the number 2 position of the indole ring.

TABLE 2. STIMULATORY EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE AND ITS DERIVATIVES. Incidence of stimulation is expressed as number of fluke preparations stimulated. Total number of fluke preparations tested at every concentration is indicated between parentheses. ED_{50} was calculated according to the logit transformation of Berkson (1953). For abbreviations and chemical structure of the different derivatives see Table 1.

Compounds	Incidence of preparations stimulated at concentration (M)				ED_{50} (M)
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
LSD		20(20)	6(10)	2(10)	$4{\cdot}8 \times 10^{-8}$
LPD		10(10)	4(10)	4(10)	$5{\cdot}5 \times 10^{-8}$
DAM		10(10)	4(10)	2(10)	$1{\cdot}3 \times 10^{-7}$
MLD		8(10)	5(10)	1(10)	$1{\cdot}3 \times 10^{-7}$
LAE	6(6)	8(10)	3(10)		$2{\cdot}5 \times 10^{-7}$
LSM	6(6)	9(10)	1(10)		$4{\cdot}1 \times 10^{-7}$
UML*	10(10)	3(10)			$3{\cdot}8 \times 10^{-6}$
	10^{-3}	5×10^{-4}	10^{-4}	10^{-5}	
BOL			6(10)	4(10)	$2{\cdot}2 \times 10^{-5}$
L-LSD	6(6)	7(10)	2(10)		$2{\cdot}9 \times 10^{-4}$

* In addition to the concentrations reported here UML was tested at 5×10^{-5} M with an incidence of four out of ten preparations tested.

increase in the tone of the preparation following the addition of the drug. The force of contraction was increased from an average of 0·5 gm to approximately 2 gm within 2 min after drug addition. Frequency and amplitude of rhythmical movement were increased in frequency and amplitude and occasional deep volleys of contraction (Fig. 1).

Other LSD derivatives which are included in Table 2 had an action that is qualitatively related to that of LSD but with different stimulant potencies. Substitution of a pyrrolidide group (lysergic acid pyrrolidide, LPD), or dimethylamide group (lysergic acid dimethylamide, DAM) for the diethylamide group caused only a slight increase in ED_{50}. Furthermore, substitution of a methyl group on the indole nitrogen (methyl lysergic acid diethylamide, MLD) caused a considerable increase in ED_{50}. The presence of one ethyl group instead of two ethyl groups (lysergic acid monoethylamide, LAE) or substitution of a morpholide group (lysergic acid morpholide, LSM) on the amide nitrogen resulted in an ED_{50} five to eight times as much as that of lysergic acid diethylamide. Methysergide (UML) which has a methyl group on the indole nitrogen and a butanol group on the amide nitrogen was one of the weak stimulants to the fluke preparations with an ED_{50} of $3{\cdot}8 \times 10^{-6}$M. Concentrations of UML as high as 10^{-5}M caused only a slight rise in tone with an increase in frequency and amplitude and occasional deep volleys of concentration (Fig. 1).

It was previously reported that bromolysergic acid diethylamide (BOL) at low concentrations either causes paralysis to rhythmical movement or is an antagonist to LSD (MANSOUR, 1957). Under our present experimental conditions BOL at concentrations above 10^{-5} M caused a weak stimulatory effect on some of the preparations which did not show spontaneous rhythmical movement. The ED_{50} for this compound was approximately four hundred times as much as that of lysergic acid diethylamide (Table 2). The stimulatory effect of BOL was characterized by an increase in amplitude of contraction (Fig. 1). The increase in amplitude and of tone which was observed following the addition

of lysergic acid diethylamide was not observed with the brominated derivative. The paralysant effect of BOL (MANSOUR, 1957) was confirmed only on preparations which showed spontaneous rhythmical movement. Furthermore, BOL antagonized the stimulant effects of both LSD and serotonin.

Of the several LSD derivatives tested the levo-otatory isomer of LSD was the least effective compound in stimulating the liver fluke preparations (Fig. 1, Table 2). The prototype compound of this series, lysergic acid, was tested at concentrations up to 10^{-3} M and was found to have no effect on these preparations.

It is concluded from these experiments that any alteration in the lysergic acid diethylamide molecule results on in a decrease in the stimulant potency of these compounds. This decrease is greatest when the substitution is on the ring structure such as in the case of BOL and UML. A change from the dextro to the levo optical isomer also caused a marked decrease in the stimulant potency of LSD.

Effect of indole derivatives

A number of indole derivatives which are related to LSD were tested (Table 3) and their effects were compared with that of LSD. Harmine, an indole with reported antiserotonin action, was the most potent stimulant drug among this group with ED_{50} of $4{\cdot}7 \times 10^{-6}$ M. 5-Hydroxytryptamine and its analogue 1-benzyl-2,5-methyl-serotonin (BAS) were equally potent in stimulating the fluke preparation. 5-Hydroxytryptophan, the precursor of serotonin, and N,N-dimethylserotonin (bufotenine) had ED_{50} of $4{\cdot}9 \times 10^{-5}$ M and $1{\cdot}1 \times 10^{-4}$ M, respectively. The 4-phosphate ester of N,N-dimethyltryptamine (psilocybin) as well as 5-hydroxyindolacetic acid (5-HIAA) did not stimulate the fluke preparations at concentrations of 10^{-3} M. These results indicate that the lysergic acid diethylamide derivatives as a group are more potent stimulants than the serotonin like compounds.

DISCUSSION

The above investigation demonstrates that all derivatives of LSD exert a stimulatory effect on the liver fluke preparations. However, a significant difference in the stimulant potency of these compounds has also been demonstrated. Among the derivatives tested above, LSD is the most potent compound. This observation is in accord with data reported on the psychotomimetic action on man (ISBELL *et al.*, 1959), and the stimulant effect on the clam heart (WRIGHT *et al.*, 1962).

A rank order correlation between the effects of these derivatives on the liver flukes and those effects on the clam heart, the rat uterus and on man which have been reported is summarized in Table 4. This correlation is based on the Spearman rank correlation coefficient (SIEGEL, 1956). It can be seen that there is a striking correlation between the relative effects of these derivatives on the flukes and those on man. LSD was the most potent compound in both systems. BOL and L-LSD, which have no psychotomimetic effect in man, were the derivatives with the least effects on the liver fluke. LPD, DAM and MLD were among the most potent agents when tested either on the flukes or in man. The relative potency of LAE on the flukes and in man was of the same order. LSM, on the other hand, was more potent in man than on the fluke.

The correlation between the effects of LSD derivatives on the liver fluke and their effects on the clam heart and the rat uterus is much lower. For example, while BOL was one of the most potent antiserotonin agents on the rat uterus it was one of the weak

TABLE 3. STIMULATORY EFFECT OF SEROTONIN AND ITS DERIVATIVES. Incidence of stimulation is expressed as in Table 1. The following names and abbreviations are used: harmine, 7-methoxy-1-methyl-9-pyrid 3,4-b indole; serotonin, 5-hydroxytryptamine; BAS, 1-benzyl-2,5-dimethylserotonin; bufotenine, 3-(2-dimethylamineoethyl)-5-indolol; psilocybin,4-phospho-N,N dimethyltryptamine; 5-HIAA, 5-hydroxy indole acetic acid.

Compounds	Incidence of flukes stimulated at concentration (M)						ED_{50} (M)
	10^{-3}	5×10^{-4}	10^{-4}	5×10^{-5}	10^{-5}	10^{-6}	
Harmine		2(2)	3(3)	9(9)	3(5)	0(2)	$4{\cdot}7\times10^{-6}$
Serotonin	5(5)	12(12)	7(10)	8(11)	3(10)	1(6)	$1{\cdot}5\times10^{-5}$
BAS			4(4)		2(4)	0(6)	$1{\cdot}5\times10^{-5}$
5-Hydroxy-tryptophan	6(7)	8(8)	4(6)		1(12)		$4{\cdot}9\times10^{-5}$
Bufotenine	6(6)	5(7)	2(4)				$1{\cdot}1\times10^{-4}$
Psilocybin	0(3)	0(3)					—
5-HIAA	0(4)						—

TABLE 4. RELATIVE POTENCIES OF LSD DERIVATIVES AND OTHER INDOLES RANKED IN DIFFERENT TEST SYSTEMS.

Compounds	Test system: Flukes	Man*	Rat uterus†	Clam Heart‡
LSD	1·0	1·0	3	1
LPD	2·0	4·5	6	
DAM	3·5	4·5	4	
MLD	3·5	2·0	1	6
LAE	5·0	6·0	5	3
LSM	6·0	3·0	7	
UML	7·0			10
Harmine	8·0			
Serotonin	9·5			4
BAS	9·5			
BOL	11·0	7·0	2	7
5-Hydroxytryptophan	12·0			9
Bufotenine	13·0			2
1-LSD	14·0	8·0	8	
Lysergic acid	15·5			5
5-HIAA	15·5			8
Rank order correlation with fluke data		0·768	0·365	0·476
Significance level, P		$<0{\cdot}05$	$>0{\cdot}2$	$>0{\cdot}1$

* Ranked on the basis of the dose of each drug which produced psychotomimetic effects in man equivalent to 1μg LSD/kg (ISBELL *et al.*, 1959).

† Ranked on the basis of the dose of each drug which inhibits by one-half a maximal contraction caused by serotonin (CERLETTI and DOEPFNER, 1958).

‡ Ranked on the basis of minimum molar concentrations of each drug which stimulated the heart of *Venus mescenaria* (GREENBERG, 1960, and WRIGHT, *et al.*, 1962).

stimulants on the liver fluke and on the clam heart. Furthermore, LPD was among the most potent stimulants to the liver fluke yet it was reported to have a low potency as an anti-serotonin compound on the rat uterus. Although the potency of MLD on the flukes and on the rat uterus was nearly of the same order, this drug had a weak effect on the clam heart. LAE, which was second only to LSD as a stimulant to the clam heart, was moderately stimulant to the fluke.

Since there is a high degree of correlation between the psychotomimetic effects of lysergic acid diethylamide on man and their actions on the rhythmical movement of the liver fluke, the biochemical effects of these compounds on these lower organisms deserve special study. The similarity between the effects of lysergic acid diethylamide and serotonin must be pointed out here. It was reported before that LSD (10^{-6} M) and serotonin (5×10^{-4} M) caused a marked increase in glycolysis in the liver fluke (MANSOUR, 1959). BOL produced no change in glycolysis when present alone but antagonized such increase induced by LSD or serotonin. Furthermore, both serotonin and LSD increased the production of cyclic 3′,5′-AMP in cell free extracts of the liver fluke (MANSOUR *et al.*, 1960). The presence of metabolic effect on mammalian tissues similar to those reported in the flukes is to be determined by further studies.

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Résumé—Les dérivés de l'acide lysergique diéthylamide et certains composés indoliques apparentés à la sérotonine ont été testés sur des préparations de douves de foie *Fasciola hepa)ica*, attachées à un dynamomètre électronique)"force transducer"). Les concentrations nécessaires pour stimuler 50% des préparations)ED_{50}) ont été déterminées.

Salon leur ED_{50}, ces dérivés peuvent être classés dans lîordre croissant: acide D-lysergique diétthylamide, acide D-lysergique pyrrolidide, acide D-lysergique dimethylamide, acide methyl-D-lysergique diethylamide, acide D-lysergique monoethylamide, acide D-lysergiquc morpholidide, acide L-methyl-D-lysergique butanolamide, acide bromo-D-lysergique diethylamide, et acide L-lysergique diethylamide.

Tous ces composés, à l'exception de l'acide bromo-D-lysergique diethylamide, ont une ED_{50} inférieure à ses dérivés apparentés.

Une bonne corrélation existe entre le pouvoir stimulant de ces agents sur la douve du foie et les effets hallucinogènes enregistrés chez l'homme.

Zusammenfassung—Lysersäurediäthylamid Derivate und eine Anzahl von Indolbindungen, ähnlich wie Serotonin, wurden an Präparationen von *Fasciola hepatica*, welche mit einer Transduktionsapparat verbunden wurden, ausprobiert, und die Konzentration, wleche 50% der Präparate stimulierte (ED_{50}), bestimmt. Diese Derivate können in einer aufsteigenden Reihe, im Bezug ihres ED_{50} in folgender Weis eangeordnet werden: D-Lysersäurediäthylamid, D-Lysersäurepyrrolidid, D-Lysersäuredimethylamid, methyl-D-lysersäurediäthylamid, D-Lysersäuremonoäthylamid, D-Lysersäuremorpholid, L-methly-D-Lysersäurebutanolamid, Bromo-D-lysersäurediäthylamid, and L-Lysersäurediäthylamid.

Alle diese Verbindungen mit Ausnahme von Bromo-D-Lyseräurediäthylamid und L-Lysers äurediäthylamid hatten einen niederigeren ED $_{50}$ als Serotonin und seine ännlichen Derivate.

Unsere Versuche zeigten eine gute Übereinstimmung des stimulierenden Effektes dieser Präparate auf den *Fasciola hepatica* und der Hervorrufung von Halluzinationen im Menschen.

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