Brit. J. Pharmacol. (1957), **12**, 406.

THE EFFECT OF LYSERGIC ACID DIETHYLAMIDE, 5-HYDROXYTRYPTAMINE, AND RELATED COMPOUNDS ON THE LIVER FLUKE, *FASCIOLA HEPATICA*

BY

T. E. MANSOUR

From the Department of Pharmacology, Louisiana State University, School of Medicine, New Orleans, Louisiana, U.S.A.

(RECEIVED APRIL 9, 1957)

The rhythmical activity of the liver fluke, *Fasciola hepatica*, was stimulated by 5-hydroxytryptamine and by lysergic acid diethylamide at very low concentrations. The effect was peripheral and was not mediated through the central ganglion. Other amines also stimulated rhythmical activity, the most potent being the indolamines.

Bromolysergic acid diethylamide, and other analogues such as yohimbine, harmine, and dopamine, depressed rhythmical movement and antagonized the stimulant action of 5-hydroxytryptamine and lysergic acid diethylamide. Evidence which suggests the presence of tryptamine receptors in the trematode is discussed.

In a previous investigation it was reported by Chance and Mansour (1949) that the liver fluke, *Fasciola hepatica*, can be used as a preparation for studying the effect of drugs on trematodes. It was found that the tone, rate, and amplitude of muscular contractions of the parasite were stimulated by amphetamine. Recent investigations by Welsh (1953) suggest that 5-hydroxytryptamine (5-HT) is a mediator of nerve action in certain invertebrates. Erspamer (1954) demonstrated the presence of 5-HT and related indolalkylamines in many invertebrate tissues. In view of these recent developments and in an attempt to obtain a better understanding of muscular activity of trematodes, it was decided to test the effect of 5-HT and of related amines on the activity of the liver fluke. In addition, observations were made upon the effect of lysergic acid derivatives on the movements of the parasite.

METHODS

The method of collecting and keeping liver flukes for the study of their movement has been described in a previous paper (Chance and Mansour, 1949). In addition to recording the movements of the intact parasite on a kymograph, a nerve muscle preparation was made in the following way. The oral end of the parasite in front of the posterior sucker was cut off, thus eliminating the effects of the circumoesophageal ganglion. The remainder of the body was then divided longitudinally into two halves. Each half was attached to a recording lever writing on a kymograph in the same way as the intact parasite preparation. The tension of the lever was 0.3 g. The preparations were completed as quickly as possible in a glass petri dish with a waxed bottom, containing Ringer solution at 37°. When mounted in a 10 ml. organ bath at 38°, these preparations did not show rhythmical activity. They will be referred to as "nerve-muscle" preparations in the text because each one consists of two layers of exposed muscle without any ganglionic control. Recording was stopped during addition of a drug to the organ bath. After addition of the drug in 0.1 ml. of solution or less, the medium was stirred by bubbling air for 1 min. to ensure uniform distribution of the compound. Solutions of drugs were adjusted to the *p*H of the medium before addition. Drugs were tested initially at a high concentration and, if active, the minimum effective concentration was determined with at least 4 preparations. The composition of the Ringer solution used was NaCl 9.0 g., KCl 0.4 g., $CaCl_2$ 0.06 g., $NaHCO_3$ 0.5 g., glucose 0.5 g./l.

RESULTS

Effect of Lysergic Acid Diethylamide and 5-Hydroxytryptamine.—Both lysergic acid diethylamide (LSD) and 5-HT stimulated the rhythmical activity of the liver fluke preparations. Concentrations of LSD higher than 10^{-7} M produced a transient increase in the tone and a great increase in amplitude of rhythmical movement of the intact parasite (Fig. 1*a*). When added to the nerve-muscle preparations at concentrations higher than 10^{-7} M, LSD initiated contractions of high ampli-

tude (Fig. 1*c*). It was observed that LSD at concentrations of 5×10^{-8} to 5×10^{-9} M caused a decrease in the rate, amplitude, and tone. This effect was followed by an increase in tone (Fig. 1*b*). The minimum concentration of 5-HT which produced stimulation was 5×10^{-5} M for the intact parasite preparation and 2.5×10^{-5} M for the nerve-muscle preparation. The stimulant effect of 5-HT on intact parasite preparations was characterized by an increase of tone, rate, and amplitude (Fig. 2). The degree of stimulation was greater in intact parasite preparations which showed weak spontaneous rhythmical movement. The response of the nerve-muscle preparation to both LSD and to 5-HT indicates that stimulation can occur in the absence of the central ganglion of the parasite.

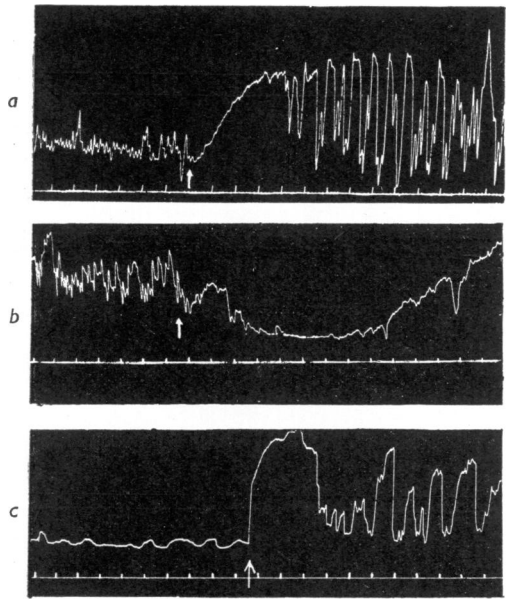

FIG. 1.—Liver fluke. Effect of lysergic acid diethylamide. In all tracings the upward stroke represents contraction recorded on a kymograph. Time markings are in min. The drugs were added at the points indicated by the arrows. (*a*) LSD, 10^{-7} M, on intact parasite preparation, (*b*) LSD $.5 \times 10^{-9}$ M, on intact parasite preparation, (*c*) LSD, 2×10^{-7} M, on nerve-muscle preparation.

Stimulant Effect of Other Amines.—Other indole derivatives and amines were tested on both preparations in order to observe any correlation between chemical structure and stimulant activity. Substances listed in Table I stimulated the intact parasite as well as the nerve-muscle preparation, indicating that the stimulant effect is peripheral and is not mediated by the central ganglion. The stimulant effect of these compounds was similar to that produced by 5-HT. Among the indolamines, 5-HT stimulated the preparations at a lower molar concentration than tryptamine, bufotenine, or 1-benzyl-5-methoxy-2-methyltryptamine. 5-Hydroxytryptophan stimulated both preparations less than did the indolamines. The stimulant effect of this compound was observed only after 5 to 10 min. Possibly the stimulant effect of 5-hydroxytryptophan is caused by a metabolic product rather than by the drug itself. The benzylamines, amphetamine, ephedrine, mephentermine, and mescaline, stimulated both the intact parasite and the nerve-muscle preparation, but at higher concentrations than did the indolamines.

TABLE I
POTENCY OF STIMULANT COMPOUNDS

Compound	Min. Effective Molar Conc.	
	Intact Prep.	Nerve-muscle Prep.
Lysergic acid diethylamide	10^{-7}	10^{-7}
5-Hydroxytryptamine	$5{\cdot}0 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$
Tryptamine	$5{\cdot}0 \times 10^{-4}$	5×10^{-4}
Bufotenine	$2{\cdot}5 \times 10^{-4}$	5×10^{-4}
1-Benzyl-5-methoxy-2-methyltryptamine	10^{-4}	10^{-4}
5-Hydroxytryptophan	4×10^{-3}	4×10^{-3}
Amphetamine	3×10^{-4}	$1{\cdot}5 \times 10^{-4}$
N-Methylphenyltertiary butylamine (mephentermine)	6×10^{-4}	6×10^{-4}
Ephedrine	10^{-3}	10^{-3}
3: 4: 5-Trimethoxyphenylethylamine (mescaline)	5×10^{-3}	5×10^{-4}

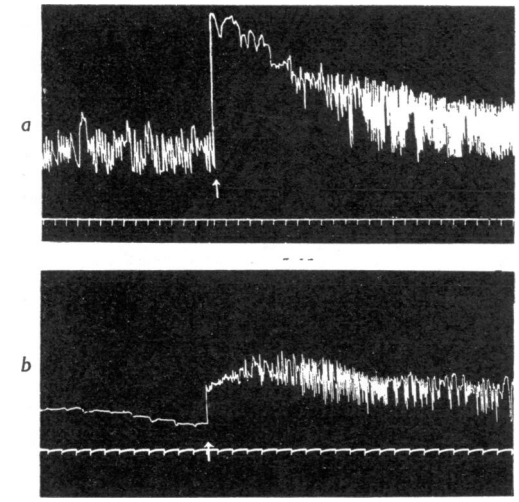

FIG. 2.—Liver fluke. Recordings as in Fig. 1. Effect of 5-hydroxytryptamine, 5×10^{-4} M, (*a*) on intact parasite preparation, (*b*) on nerve-muscle preparation.

Drugs which Inhibited Rhythmical Activity.—Substances listed in Table II depressed the rhythmical activity of the intact parasite preparations

TABLE II

DRUGS WHICH DEPRESSED RHYTHMICAL ACTIVITY OF INTACT PARASITE PREPARATIONS

Drugs	Min. Effective Molar Conc.
Bromolysergic acid diethylamide ..	10^{-7}
Yohimbine	10^{-4}
Harmine	5×10^{-4}
3:4-Dihydroxyphenylethylamine (dopamine)	10^{-3}

sometimes to the point of complete paralysis. These compounds did not have any effect on the nerve-muscle preparations. Bromolysergic acid diethylamide (BOL) was the most potent drug, depressing the intact parasite preparation at concentrations above 10^{-7} M (Fig. 3). Yohimbine at

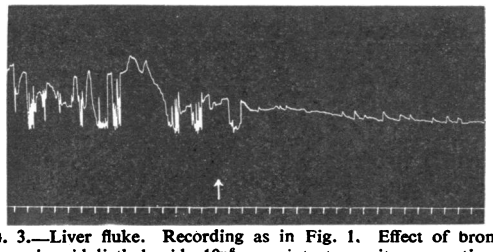

FIG. 3.—Liver fluke. Recording as in Fig. 1. Effect of bromolysergic acid diethylamide, 10^{-6} M, on intact parasite preparation.

concentrations higher than 10^{-4} M and harmine at concentrations higher than 5×10^{-4} M depressed rhythmical activity. 3:4-Dihydroxyphenylethylamine (dopamine), which has been shown to be rapidly oxidized by a phenol oxidase in these parasites (Mansour, 1957), inhibited rhythmical activity at concentrations higher than 10^{-3} M.

Antagonistic Effect of Stimulatory and Inhibitory Drugs.—The effects of 5-HT, LSD, and amphetamine were tested on both preparations in the presence of various concentrations of BOL. It was found that BOL antagonized the effect of these stimulants. Furthermore, the depressant effect of BOL on the intact parasite preparation did not occur in the presence of the stimulant drugs (Figs. 4 and 5). Table III gives the concentrations of BOL and various stimulant drugs which, when added together, did not affect significantly the motility of either preparation. When the concentration of the stimulant was increased, it was necessary to increase the concentration of the antagonist to overcome the stimulation. For example, BOL in a concentration of 10^{-6} M antag-

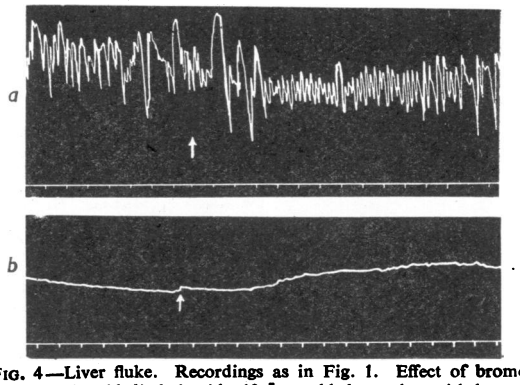

FIG. 4.—Liver fluke. Recordings as in Fig. 1. Effect of bromolysergic acid diethylamide, 10^{-7} M, added together with lysergic acid diethylamide, 10^{-7} M, (*a*) on intact parasite preparation, (*b*) on nerve-muscle preparation.

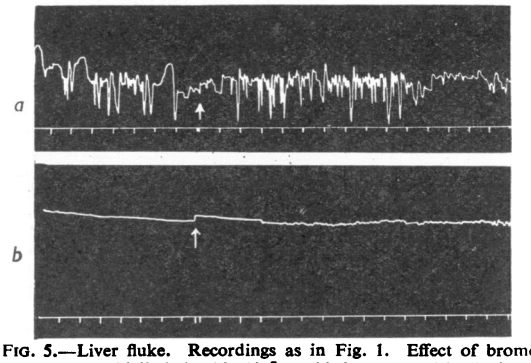

FIG. 5.—Liver fluke. Recordings as in Fig. 1. Effect of bromolysergic acid diethylamide, 10^{-7} M, added together with 5-hydroxytryptamine, 5×10^{-4} M, (*a*) on intact parasite preparation, (*b*) on nerve-muscle preparation.

TABLE III

ANTAGONISM OF BROMOLYSERGIC ACID DIETHYLAMIDE TO STIMULANT DRUGS

The antagonistic BOL concentrations were determined with at least 4 preparations.

Stimulant Drug (Molar Conc.)	Antagonistic BOL Molar Conc.	
	Intact Prep.	Nerve Muscle Prep.
5-Hydroxytryptamine 5×10^{-4}	10^{-6}	10^{-5} to 10^{-6}
,, 10^{-3}	10^{-5}	10^{-4}
Lysergic acid diethylamide 10^{-7}	10^{-7}	10^{-7}
,, ,, ,, 10^{-6}	10^{-6}	10^{-6}
Amphetamine 5×10^{-4} ..	10^{-6}	10^{-6}
,, 10^{-3} ..	10^{-5}	10^{-5}

onized the stimulant effect of 5×10^{-4} M 5-HT. The concentration of BOL required to antagonize 10^{-3} M 5-HT was 10^{-5} M. The same phenomenon was observed when LSD or amphetamine was used as a stimulating agent. The concentration of BOL required to antagonize amphetamine was the same as that which antagonized 5-HT. Yohimbine

(10^{-4}) inhibited the stimulant effect of 5×10^{-4} M 5-HT on the intact preparation and 10^{-4} M on the nerve-muscle preparation. Dopamine (10^{-4} M) inhibited the stimulatory effect of 10^{-4} M 5-HT on both the intact and the nerve-muscle preparations. Harmine (5×10^{-4} M) did not interfere with the stimulant effect of 5-HT on either preparation.

Ineffective Compounds.—(−)-Adrenaline, (−)-noradrenaline, histamine, chlorpromazine, and sodium bromide at concentrations of 5×10^{-3} M had no effect on either preparation. The absence of any effect of sodium bromide indicates that the bromide ion does not interfere with rhythmical movement.

Discussion

The present study has shown that 5-HT stimulates muscular contraction of the trematode *Fasciola hepatica*. Furthermore, rhythmical movement in isolated muscle strips of the parasite was initiated by 5-HT, which indicates that the action of this substance is mediated by peripheral receptors. The powerful stimulant activity of 5-HT on these helminths and the fact that 5-HT occurs normally in many invertebrates (Erspamer, 1954) raise the possibility that this or a related compound may be the humoral transmitter for the peripheral receptors of *Fasciola*. Other chemical mediators such as histamine, adrenaline, or noradrenaline do not have any effect on the parasite.

Movement in the intact liver fluke can be depressed by BOL, harmine, and yohimbine, all of which contain the indole structure and may be looked upon as complex analogues of 5-HT. Possibly the depressant compounds act by combining with tryptamine receptors, thus blocking the effect of endogenously released transmitter. This hypothesis is supported by the fact that BOL antagonized the stimulant effects of 5-HT, LSD, and amphetamine on intact preparations. The stimulant effect of these compounds on nerve-muscle preparations was also inhibited by BOL, which, by itself, had no effect. Furthermore, it was found necessary to increase the concentration of the stimulant whenever the concentration of the depressant drug was increased. The similarity between the effects of amphetamine and 5-HT and the fact that BOL antagonized both compounds suggest that these amines may act on the same receptors. The fact that LSD inhibited rhythmical activity of intact parasite preparations at concentrations lower than its stimulant potency cannot yet be explained.

It is of interest to note that LSD acted on muscular activity in the same manner as 5-HT. Both compounds affect the metabolism of the liver fluke in a similar manner (Mansour, 1956). Recent reports by Shaw and Woolley (1956), and Welsh and McCoy (1957), indicate that LSD has activity similar to that of 5-HT in many vertebrate as well as invertebrate tissues. In the instance of *Fasciola hepatica*, LSD, on a molar basis, was a much more potent stimulant than 5-HT itself; this may be due to a greater affinity of the receptors for LSD. Certain analogues have been found to be potent antagonists of 5-HT and of LSD. Sollero, Page, and Salmoiraghi (1956) have recently demonstrated that BOL is a very potent antagonist to 5-HT on sensitized rat uterus. Ginzel and Mayer-Gross (1956) have made the observation that BOL inhibits LSD psychosis in man. The inhibitory effect of BOL to the action of LSD and of 5-HT in *Fasciola* appears to parallel its action on mammals. These findings support the hypothesis that the pharmacological effect of LSD and BOL could be attributed to the fact that these compounds are structural analogues of 5-HT.

I am grateful to Professor Ernest Bueding for his constant help and suggestions. My thanks are also due to Mr. A. D. Lago and Miss Carol Fitzpatrick for technical assistance. I gratefully acknowledge the gift of substances used in this investigation from the following: Dr. D. W. Woolley, of the Rockefeller Institute; Dr. R. Heinle, of the Upjohn Company; Dr. R. Bircher, of Sandoz Pharmaceuticals; Dr. Joseph Seifter, of Wyeth Laboratories; and Dr. Paul Mattis, of Smith, Kline and French.

This work was supported by research grants from the U.S. Public Health Service (Nos. E-1386 and E-668) and from Swift and Co.

References

Chance, M. R. A., and Mansour, T. E. (1949). *Brit. J. Pharmacol.*, **4**, 7.
Erspamer, V. (1954). *Pharmacol. Rev.*, **6**, 425.
Ginzel, K. H., and Mayer-Gross, W. (1956). *Nature, Lond.*, **178**, 210.
Mansour, T. E. (1956). *Fed. Proc.*, **15**, 454.
—— (1957). *J. Pharmacol.*, **119**, 164.
Shaw, E., and Woolley, D. W. (1956). *Science*, **124**, 121.
Sollero, L., Page, I. H., and Salmoiraghi, J. (1956). *J. Pharmacol.*, **117**, 10.
Welsh, J. H. (1953). *Anat. Rec.*, **117**, 637.
—— and McCoy, A. C. (1957). *Science*, **125**, 348.