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BIOCHEMICAL EFFECTS OF LYSERGIC ACID DIETHYLAMIDE ON THE LIVER FLUKE *FASCIOLA HEPATICA**

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Abstract—Serotonin (5-hydroxytryptamine) and lysergic acid diethylamide cause an increase in glycolysis in the liver fluke *Fasciola hepatica*. The effect of serotonin on glycolysis appears to be due to an increase in phosphofructokinase activity and is mediated through cyclic 3′,5′-AMP. The biochemical effects of lysergic acid diethylamide on the liver flukes were examined in the present paper. Like serotonin, lysergic acid diethylamide causes an increase in the activity of phosphofructokinase in the liver flukes. This effect can be obtained either by preincubating the intact flukes with lysergic acid diethylamide or by adding it directly to fluke homogenates. While serotonin activates phosphofructokinase through its effect on a particulate fraction from the flukes, lysergic acid diethylamide did not have an effect on this fraction. The question whether lysergic acid diethylamide effect is mediated through serotonin was investigated. The concentration of serotonin in intact liver flukes was found to be 231 ng/g wet weight. The level of serotonin in the flukes is markedly increased when the flukes are incubated with 5-hydroxytryptophan but is unchanged following incubation with tryptophan. Lysergic acid diethylamide does not change the levels of endogenous serotonin in control flukes. It also does not affect the levels of serotonin synthesized by the organism from 5-hydroxytryptophan. The data does not support the idea that the effect of lysergic acid diethylamide is mediated through an increase in serotonin levels although the effects on carbohydrate metabolism of both agents are similar.

PREVIOUS investigations indicated that serotonin (5-hydroxytryptamine) has a function in the liver fluke similar to that of epinephrine in higher organisms. Serotonin increased glycogen breakdown, lactic acid production,[1] glycogen phosphorylase activity,[2] glucose utilization,[1] phosphofructokinase activity[3] and the production of cyclic 3′,5′-AMP.[2] Serotonin and other related indolalkylamines increased rhythmical activity of the liver fluke.[4] The question whether the biochemical effects observed in intact organisms with serotonin are secondary to the effects on rhythmical movement was resolved when it was found that the indolalkylamine, when added directly to cell-free extracts from the parasites, can reproduce all the effects observed on intact organisms. Thus, serotonin appears to be directly responsible for these effects on the metabolism of intact organisms.

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Lysergic acid diethylamide (LSD) is an agent which has always been connected pharmacologically with serotonin. Although LSD antagonizes the effect of serotonin on mammalian smooth muscle it was found to reproduce the effect of serotonin on rhythmical activity of the liver flukes.[4] The latter effect can be demonstrated by LSD at concentration levels which are at least 100-fold less (10^{-7} M) than those of serotonin. The present investigation was carried out in an attempt to examine the effect of LSD on some of the biochemical systems which respond to serotonin. Some biochemical effects of LSD which have already been reported include stimulation of glycolysis and glycogenolysis[1] as well as a slight increase in the production of cyclic 3′,5′-AMP by particulate fractions.[2] The question whether the effects of LSD are mediated through an increase in the endogenous serotonin levels was also investigated. Furthermore, some experiments concerning serotonin synthesis in intact organisms will be reported. The findings indicate that although LSD can reproduce many of the effects of serotonin, these effects cannot be attributed to a net increase in the levels of serotonin in intact organisms. During our investigation we were guided by the studies of the late Dr. Nicholas Giarman[5–7] to whose memory we are dedicating this paper.

MATERIALS AND METHODS

The liver flukes, *Fasciola hepatica*, were obtained from the bile ducts of infested cattle at a local slaughterhouse. The worms were transported and maintained in the laboratory as described previously.[1] Assays of phosphofructokinase activity in fluke homogenates were carried out as described before.[1]

Determination of serotonin was carried out on batches of four to five flukes (200 to 450 mg). The flukes were rinsed in distilled water, dried on filter paper and frozen on a pair of Wallenberger tongs which had previously been cooled on dry ice. The frozen flukes were then weighed and homogenized in 7 ml of ice-cold 0·1 N HCl. Serotonin was extracted from the homogenates by the alkaline method of Bogdanski *et al.*,[8] with the exception that in place of 0·1 N HCl, a final aqueous phase of 1·4 ml of 0·05 M phosphate buffer, pH 7·0, was used. In flukes which were incubated with tryptophan or 5-hydroxytryptophan, the butanol phase was washed three times with borate buffer, pH 10, to insure removal of those interfering amino acids. The extracted serotonin was measured fluorometrically after reacting with ninhydrin by the method of Snyder *et al.*[9] Since extraction of serotonin is approximately 70 per cent, serotonin levels of flukes were calculated from the fluorescence of known quantities of serotonin carried through the same extraction procedure. Serotonin added to fluke homogenates and extracted by the same procedure was recovered to the same degree as control samples. Determination of serotonin levels by this procedure in the brain of adult Sprague–Dawley rats gave a value of 470 ng/g wet weight which is within the range of normal values reported before.

Chemicals and drugs were obtained from the same sources reported before.[3]

RESULTS

Activation of phosphofructokinase by lysergic acid diethylamide (LSD)

It was previously reported that phosphofructokinase in fresh liver flukes was nearly inactive when tested under conditions described in Table 1.[3, 10, 11] Incubation of the flukes with serotonin caused activation of the enzyme. Serotonin, as well as cyclic 3′,5′-AMP, when added directly to cell-free extracts of the flukes also activated the

enzyme. The results summarized in Table 1 show that, as in the case of serotonin, LSD caused a marked activation of this enzyme when added to the fluke incubation medium. Since LSD at the concentration tested increased the activity of these worms the question arose whether the effect is secondary to an increase in muscular activity or whether it results from a direct action of LSD. The results in Table 2 show that LSD when added directly to cell-free extracts of the flukes caused an increase in enzyme activity. The degree of activation by serotonin and by cyclic 3′,5′-AMP is also shown in Table 2. These experiments indicate that LSD when added directly to fluke extracts is much less active than when the flukes were preincubated with the drug. Furthermore, LSD has a smaller stimulating action on the enzyme than serotonin or cyclic 3′,5′-AMP.

TABLE 1. EFFECT OF LYSERGIC ACID DIETHYLAMIDE TARTRATE (LSD) ON PHOSPHOFRUCTOKINASE ACTIVITY IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Additions to fluke incubation medium	Phosphofructokinase activity (μmole fructose-1,6-di-P per g wet wt.)
None	0·7
None	0·7
10^{-5} M LSD	12·3
10^{-5} M LSD	12·5

Flukes were incubated for 2 hr (as described before[3]) in the regular phosphate Ringer solution with the indicated additions. After incubation, flukes were rinsed with distilled water and homogenized in 0·01 M cold potassium glycylglycine buffer, pH 7·5 (1 g/10 ml). Phosphofructokinase assays were carried out in 0·05 M glycylglycine buffer, pH 7·5; 0·008 M $MgCl_2$; 0·01 M ATP and 0·005 M fructose-6-P. The amount of fructose-1,6-di-P was determined enzymatically after incubation for 10 min at 30°. For further experimental details see methods published before.[3]

TABLE 2. DIRECT EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON PHOSPHOFRUCTOKINASE IN CRUDE HOMOGENATES FROM THE LIVER FLUKE *FASCIOLA HEPATICA*

Additions to the reaction mixture	Phosphofructokinase activity (μmole fructose-1,6-di-P per g wet wt.)
None	0·9
10^{-4} M LSD	4·9
10^{-5} M LSD	4·5
10^{-6} M LSD	3·5
None	0·7
5×10^{-4} M serotonin	19·6
5×10^{-5} M serotonin	20·6
5×10^{-6} M serotonin	9·2
7×10^{-5} M cyclic 3′,5′-AMP	24·0

Flukes were homogenized in 0·01 M cold glycylglycine buffer, pH 7·5. Phosphofructokinase was assayed as described in Table 1.

Previous investigations on the nature of activation of fluke phosphofructokinase by serotonin and by cyclic 3′,5′-AMP showed that an inactive form of the enzyme can be isolated by a procedure involving differential centrifugation.[11] The inactive enzyme (150,000 *g*-sediment) can be activated by serotonin only in the presence of another cell fraction (80,000 *g*-sediment) with ATP and Mg^{2+}. Serotonin when incubated with the 80,000 *g*-sediment, ATP, and Mg^{2+} gives a thermostable fraction which can activate phosphofructokinase in the 150,000 *g*-sediment. The effect of LSD was tested on this system and compared with that of serotonin and cyclic 3′,5′-AMP. Results in

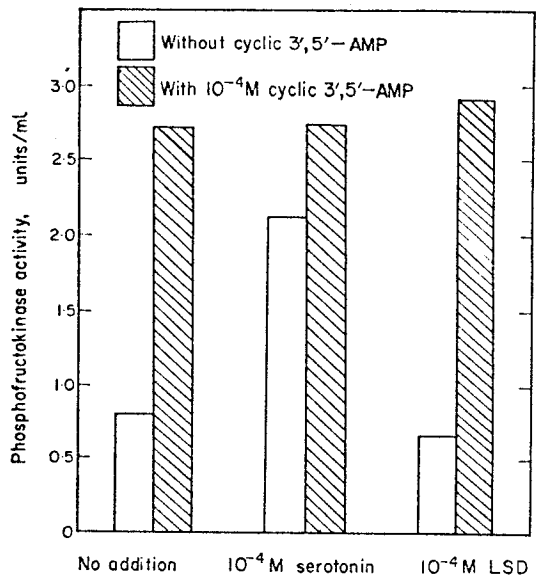

FIG. 1. Effect of serotonin and lysergic acid diethylamide (LSD) on the ability of the 80,000 *g* sediment from fluke homogenates to activate phosphofructokinase. Thermostable fractions were prepared by incubation of the 80,000 *g*-sediment from the fluke homogenates with ATP, Mg^{2+}, caffeine 0·013 M and the additions indicated on the abscissae of the figure (as described before[11]). Thermostable fractions obtained from these incubations were used for enzyme activation in a system containing 0·02 ml 150,000 *g*-sediment and 0·065 ml thermostable fraction in a final volume of 0·1 ml. Striped bars indicate the addition of 10^{-4} M cyclic 3′,5′-AMP to the enzyme activation system. After incubation for 12 min at 30°, the activation mixture was diluted 200-fold and assayed for phosphofructokinase activity spectrophotometrically by coupling the enzyme to aldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase. For further experimental details see "Material and Methods" of previous publication.[11] Results are expressed as units of phosphofructokinase activity per ml of 150,000 *g*-sediment.

Fig. 1 show that thermostable fraction prepared with serotonin present caused activation while fraction prepared with LSD did not affect phosphofructokinase activity. The effect of cyclic 3′,5′-AMP was observed under any of these experimental conditions. It appears therefore that in this system, unlike in the case of other biochemical parameters tested, LSD does not have the same effect as serotonin.

Effect of LSD on serotonin levels in the liver fluke

The close similarities between the effects of both serotonin and of LSD on the

intact organisms have suggested that LSD could act through an increase in the levels of serotonin in the parasites. To test this hypothesis the levels of serotonin in control as well as LSD-treated flukes were determined. Preliminary experiments[12] using a bioassay method for the determination of serotonin showed that the flukes contain a serotonin-like material and a system for its synthesis. Determination of serotonin by a fluorometric procedure was carried out in control flukes and is summarized in Table 3.

TABLE 3. THE PRESENCE OF SEROTONIN IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Exp. no.	Serotonin content (ng/g wet wt.)	
	Individual determinations	Average
1	191 226	208
2	261 355 136 172 137	212
3	176 181 185	181
4	263 274	268
5	191 215	203
6	319	319
7	139 324 204 210	219
8	237	237
9	251 219	235
10	183 310 210	234
11	265	265
12	205 227 160	197
	Mean	231
	Standard deviation	±38
	Standard error	±11

Individual determinations represent serotonin content in separate batches of flukes.

TABLE 4. EFFECT OF LYSERGIC ACID DIETHYLAMIDE (LSD) ON THE LEVELS OF SEROTONIN IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Additions to fluke incubation medium	Serotonin content (ng/g wet wt.)	
	Individual determinations	Average ± S.D.
Exp. 1		
None	183 310 210	234 67
10^{-4} M LSD	229 203 246	226 22
Exp. 2		
None	261 355	308
10^{-5} M LSD	325 299 289	337 56
Exp. 3		
None	191 226	208
10^{-5} M LSD	196 179	187
10^{-6} M LSD	197 406	301
Exp. 4		
None	263 274	268
10^{-6} M LSD	269 299	284
Exp. 5		
None	176 181 185	181 5
10^{-7} M LSD	198 166	182

Flukes (four to five per batch) were incubated for ½ hr in 25 ml of fluke saline solution containing 1 mg of dextrose per ml at 37°. Individual determinations represent serotonin content in separate batches of flukes.

For convenience the data from all control experiments were pooled and a mean value of 231 ng per g wet weight of flukes was obtained. These values are about twice what we have reported before in our preliminary determinations using acetone extraction and assays on sensitized rat uterus. The difference might be attributed to the more efficient present method of extraction and to a greater accuracy of the fluorometric method for serotonin determination.

The effect of LSD on the levels of serotonin in the liver flukes is summarized in Table 4. The results show that lysergic acid diethylamide at concentrations which stimulate rhythmical movement of the parasite and cause all the above-described biochemical changes (10^{-4}–10^{-7} M) caused no significant change in the level of serotonin in this organism.

Effect of LSD on the synthesis of serotonin from 5-hydroxytryptophan

A preliminary demonstration[12] reported that cell-free extracts from the liver flukes can synthesize serotonin from 5-hydroxytryptophan. The question whether such a system can be demonstrated in intact organisms and whether LSD has an effect on it was investigated. The effects of different amino acids on the levels of serotonin in the flukes were also determined. Results summarized in Table 5 show that incubation of

TABLE 5. EFFECT OF DIFFERENT AMINO ACIDS ON THE LEVELS OF SEROTONIN IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Additions to fluke incubation medium	Incubation time (hr)	Serotonin content (ng/g wet wt.)	
		Individual determinations	Mean ± S.D.
Exp. 1			
None	½	176 181 185	181 4
10^{-3} 5-hydroxytryptophan	½	602 414 484	500 95
Exp. 2			
None	1	139 324 204 210	219 77
10^{-3} tryptophan	1	191 254 125	190 64
10^{-3} phenylalanine	1	106 215 178	166 55
Exp. 3			
None	15	205 227 160	197 34
10^{-3} tryptophan	15	103 111 170	128 37

Individual determinations represent serotonin content in separate batches of flukes.

the flukes with 10^{-3} M 5-hydroxytryptophan almost tripled the contents of serotonin in ½ hr. On the other hand, incubation with tryptophan or phenylalanine caused no significant change in the levels of the amine in the flukes. These results indicate that in the flukes, although serotonin can be synthesized from 5-hydroxytryptophan, the enzyme system for the conversion of tryptophan to 5-hydroxytryptophan appears either to be absent or had negligible activity under the present conditions. The increase in the level of serotonin was found to be concentration- (Fig. 2) and time-dependent (Fig. 3). Figure 3 shows that incubation of the flukes with 5-hydroxytryptophan for as long as 4 hr could raise the level of serotonin to approximately 8-fold the average levels in control flukes. The activity of the parasites incubated with 5-hydroxytryptophan was observed and was found to be increased as in the case with serotonin.

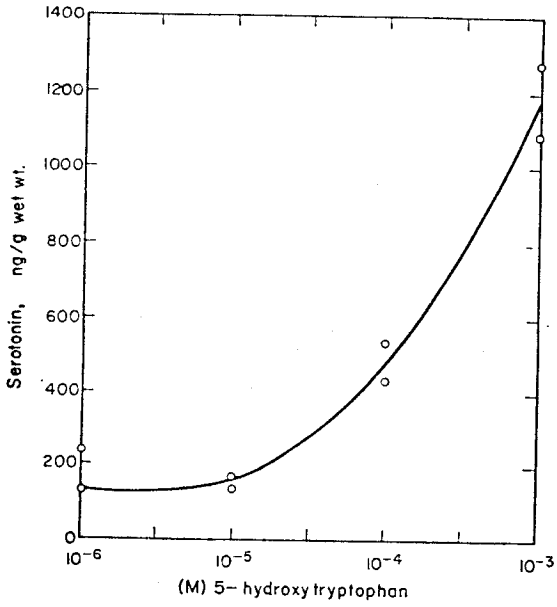

FIG. 2. Levels of serotonin in the liver flukes after incubation with different concentrations of 5-hydroxytryptophan for 75 min. Curve was drawn to connect nearly the mean values of individual determinations from the same experiment.

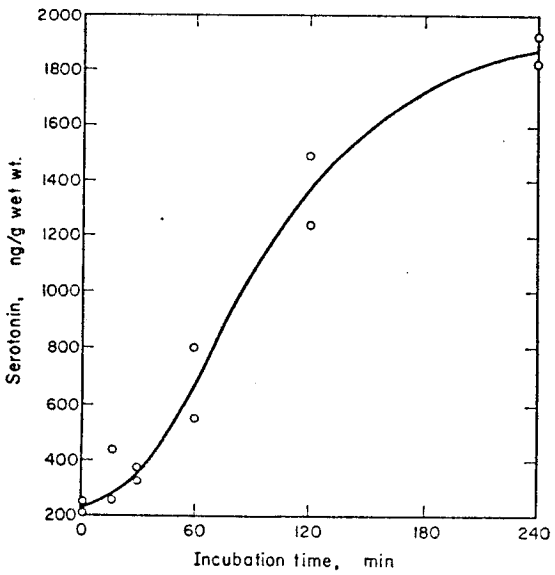

FIG. 3. Levels of serotonin in the liver flukes after incubation with 10^{-3} M 5-hydroxytryptophan for different times. Curve was drawn to connect nearly the mean values of individual determinations from the same experiment.

Furthermore, incubation of the flukes with 5-hydroxytryptophan caused an increase in glucose utilization and lactic acid production (Table 6) as was reported previously with serotonin.[4] It therefore appears that raising serotonin endogenously by providing the substrate, 5-hydroxytryptophan, affects both metabolism and motility in the same manner as does addition of serotonin.

The effect of LSD on the synthesis of serotonin from 5-hydroxytryptophan by intact liver flukes was tested. These experiments were carried out in the presence of different concentrations of 5-hydroxytryptophan. The results summarized in Table 7 show that LSD at concentrations of 10^{-4} M and 10^{-5} M did not cause a significant change of serotonin level from that of control flukes.

TABLE 6. EFFECT OF 5-HTP AND OF 5-HT ON GLUCOSE UPTAKE AND ON LACTIC ACID PRODUCTION BY THE LIVER FLUKE *FASCIOLA HEPATICA*

Additions to fluke incubation medium	Glucose removed (μmole/g wet wt.)	Lactic acid produced (μmole/g wet wt.)
None	80	16
10^{-3} M 5-HTP	94	21
10^{-4} M 5-HTP	92	ND*
10^{-3} M 5-HT	116	86
None	92	48
10^{-3} M 5-HTP	135	129
10^{-4} M 5-HTP	109	122
None†	92	32
10^{-6} M LSD†	124	63

* Not determined.
† From data reported previously.[1]
Flukes were incubated for 3 hr in saline medium containing 0·01 M glucose as described before.[1]

TABLE 7. EFFECT OF LYSERGIC ACID DIETHYLAMIDE (LSD) ON THE SYNTHESIS OF SEROTONIN FROM 5-HYDROXYTRYPTOPHAN (5-HTP) BY INTACT LIVER FLUKES (*FASCIOLA HEPATICA*)

Exp. No.	5-HTP concentration (M)	LSD concentration (M)	Serotonin content (ng/g wet wt.) Individual determinations	Average ± S.D.
1	10^{-5}	0	277 166	221
	10^{-5}	10^{-5}	157 157	157
	10^{-5}	10^{-4}	228 216	222
	10^{-4}	0	161 645	403
	10^{-4}	10^{-5}	237 255	246
	10^{-4}	10^{-4}	266 270	268
2	10^{-5}	0	307 310	308
	10^{-5}	10^{-5}	305 292	298
	10^{-5}	10^{-4}	327 305	316
3	10^{-5}	0	225 238 240	234 ± 8
	10^{-5}	10^{-5}	360 377 277	338 ± 54

Flukes (four to five per batch) were incubated for 1 hr in experiments 1 and 3 and for 2 hr in experiment 2 in 25 ml of fluke saline medium containing 1 mg dextrose per ml and the indicated additions. Individual determinations represent serotonin content in separate batches of flukes.

DISCUSSION

The results reported above show that the liver fluke *Fasciola hepatica* contains significant amounts of serotonin. The amount of serotonin in the intact organisms (231 ng per g wet weight) is approximately half of what was found in the rat brain (470 ng per g wet weight). Furthermore, there is an active system for the synthesis of the indolalkylamine from 5-hydroxytryptophan. The widespread distribution of serotonin among other invertebrates has been reported.[13–15] The biochemical and physiological effects of 5-hydroxytryptophan in the liver flukes are identical to those of serotonin and appear to be mediated through the conversion of the amino acid to serotonin. Results reported before showed that stimulation of rhythmical movement by 5-hydroxytryptophan appeared 5–10 min after addition of the amine while stimulation by serotonin appeared almost instantaneously, indicating that the amino acid is converted to serotonin before it exerts its stimulating effect.[4] Evidence has been accumulating indicating that serotonin has a function in invertebrate tissues similar to that of epinephrine in higher organisms.[15–17] This appears particularly true with the liver fluke *Fasciola hepatica*. Several of the effects of serotonin on the carbohydrate metabolism of the liver fluke appear to be analogous to the effects of catecholamines in mammalian tissues. These facts raise the question whether serotonin or a related indolalkylamine has a hormonal regulatory function in the fluke similar to that of epinephrine in higher animals.[2, 17]

The biochemical effects of LSD on the liver fluke described above appear to be identical with those of serotonin. The results, however, show that LSD does not change the levels of serotonin to a significant degree. It appears, therefore, that the effects of LSD are not mediated through an increase in serotonin levels. However, other possible mechanisms for a serotonin-mediated effect of LSD have not been excluded. These include: (a) the possibility that LSD might have an effect on the turnover rate of serotonin, and (b) LSD might cause a change in the distribution of free and bound serotonin without changing total levels of the amine, as was shown in rat brain by Giarman and his colleagues.[5–7] The possibility that LSD effect is entirely independent of serotonin is suggested in the above results by the finding that in the phosphofructokinase activation system which is sensitive to serotonin, LSD failed to show any effect. On the other hand, the enzyme, either in the intact organism or in the fluke homogenates, was stimulated by both serotonin and LSD.

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