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# Adenosine Cyclic 3',5'-Monophosphate in the Liver Fluke, *Fasciola hepatica*

## I. Activation of Adenylate Cyclase by 5-Hydroxytryptamine

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SUMMARY

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5-Hydroxytryptamine caused a marked accumulation of endogenous adenosine cyclic 3',5'-monophosphate in the liver fluke, *Fasciola hepatica*, especially in the anterior end ("head") of the organism. The accumulation of the cyclic nucleotide and the accompanying increase in motility of the isolated fluke head were dependent on 5-hydroxytryptamine concentration. Adenylate cyclase in particles from fluke heads also showed a greater activation by 5-hydroxytryptamine than did particles from the posterior end of the fluke. The ability of indoleamine analogues of 5-hydroxytryptamine to stimulate fluke motility and to activate adenylate cyclase was found to diminish with decreasing structural similarity of the analogue to 5-hydroxytryptamine. Both the free amino and 5-hydroxyl groups of 5-hydroxytryptamine appear to be important for its recognition by adenylate cyclase. The results support the hypothesis that adenosine cyclic 3',5'-monophosphate acts as a second messenger for 5-hydroxytryptamine in invertebrates. On the other hand, this investigation suggests no direct relationship between the elevation of the cyclic nucleotide and the stimulation of motility. Both amphetamine and D-lysergic acid diethylamide stimulated fluke motility but did not cause an increase in endogenous levels of the cyclic nucleotide. Moreover, D-lysergic acid diethylamide antagonized the 5-hydroxytryptamine activation of adenylate cyclase. The results suggest that this antagonism may be related to the high affinity of D-lysergic acid diethylamide for its site on adenylate cyclase.

## INTRODUCTION

5-Hydroxytryptamine appears to play a role in the regulation of carbohydrate metabolism and neuromuscular activity in the liver fluke, *Fasciola hepatica*. It stimulates glycolysis and glycogenolysis (1, 2), activates glycogen phosphorylase and phosphofructokinase (3-5), and increases rhythmical movement of this organism (6). 5-HT[3] activates adenylate cyclase [ATP: pyrophosphate-lyase (cyclizing), EC 4.6.-1.1] in the fluke, and its effects on carbohydrate metabolism appear to be mediated through cAMP (3). 5-HT is present in the fluke, and there is an active system for its

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[3] The abbreviations used are: 5-HT, 5-hydroxytryptamine (serotonin); cAMP, adenosine cyclic 3',5'-monophosphate; LSD, D-lysergic acid diethylamide.

synthesis from its metabolic precursor, 5-hydroxytryptophan (7). Incubation of the fluke with this precursor raises the level of endogenous 5-HT and thus stimulates carbohydrate metabolism and motility as well (7). This indicates that external addition of 5-HT has effects which are similar to those of internally produced 5-HT. On the basis of these findings we hypothesized that 5-HT may have a hormonal function similar to that of epinephrine in higher organisms. Such a function by 5-HT in several other invertebrates can be suggested on the basis of work by Welsh (8).

The present investigation was undertaken to study the possible relationship between activation of adenylate cyclase by 5-HT and stimulation of neuromuscular activity in the fluke. First, we attempted to answer the question whether stimulation of rhythmical movement by 5-HT is accompanied by an increase in the level of cAMP in the organism. Because cAMP has been implicated as a component of synaptic transmission, we investigated whether the increase in cAMP level is more demonstrable in those parts of the fluke that are rich in ganglionic structures. We also tested several analogues of 5-HT and studied the relationship between the ability of these compounds to stimulate fluke motility and their effect on cAMP formation. Of special interest was LSD, a compound which we previously reported to have a slight activating effect on fluke adenylate cyclase (3). Several other properties of the fluke adenylate cyclase, both in particles and in intact organisms, were investigated. In the accompanying paper (9) we correlate the effects of 5-HT and LSD on adenylate cyclase with their effects on protein kinase.

## METHODS

*Adenylate cyclase activity in fluke particles. Fasciola hepatica* were collected from fresh beef livers at a local slaughterhouse and maintained in saline medium with bovine serum and antibiotics as previously described (1). Before use in an experiment, the flukes were rinsed in fresh, warm (37°) saline medium (pH 8.8). Fluke "heads" were obtained by servering the oral end of the animal just below the posterior sucker. The remainder of the fluke is referred to as the "tail."

Particulate fractions of at least six whole flukes, 20 heads, or six tails were prepared as previously decribed (3). Suspensions of particles were used either without further dilution or after a 3-fold dilution with 0.25 M sucrose. The reaction mixture for assay of adenylate cyclase activity contained 50 mM glycylglycine (pH 7.5), 6.6 mM $MgSO_4$, 10 mM caffeine, 4 mM sodium ATP, and the indicated concentration of test substance(s). The reaction was initiated by addition of 0.4 ml of particles (bringing the total volume to 1 ml) and then incubated in a Dubnoff shaker at 37°. After the indicated time, the rection was stopped by immersion of the sample tube in a boiling water bath for 4 min. The tube was placed in ice, and 0.6 ml of cold $H_2O$ was added, followed by 0.2 ml of 0.25 M $ZnSO_4$ and enough saturated $Ba(OH)_2$ to neutralize the mixture. After 10 min the sample was centrifuged at 8000 × *g* for 15 min and the supernatant was used for determination of cAMP concentration by the protein binding method described by Gilman (10). The dried Millipore filters (HAWP 02400) were wedged firmly into the bottoms of scintillation vials and counted in 10 ml of standard toluene-based scintillation fluid. A zero-time blank for the adenylate cyclase assay was obtained by heating the particles to 98° for 4 min before addition to the reaction mixture. For calculation of net enzyme activity, the value of this blank was subtracted from all experimental values. There was no measurable cAMP in samples which were incubated in the absence of ATP.

The identity of cAMP was further verified by treatment of the sample (prior to the barium-zinc precipitation) with excess purified cAMP phosphodiesterase for 1 hr at 37°. Phosphodiesterase was a generous gift from Professor R. W. Butcher of the University of Massachusetts.

*Determination of endogenous cAMP.* For each experimental condition, 20 heads, six tails, or six whole flukes were allowed to equilibrate for a few minutes in 4.50–4.95 ml of saline medium at 37°. Test substances or water were added in a volume of

0.05–0.5 ml, and the mixture was stirred (final volume was 5.00 ml). After the indicated time, the bathing medium was drained, and the tissue was removed and immediately frozen with Wollenberger clamps which had been chilled on Dry Ice. Tissues were homogenized in a motor-driven Potter-Elvehjem homogenizer for 1 min in 0.5 ml of 0.3 N HCl. Then 40 $\mu$l of the homogenate were transferred to 0.96 ml of 1 N KOH, and the mixture was allowed to stand overnight before protein determination by the method of Lowry *et al.* (11). The remainder of the homogenate was measured and then placed in a centrifuge tube to which were added 5 $\mu$l (170 pmoles) of [$^{14}$C]cAMP (Schwarz/Mann; specific activity, 55 $\mu$Ci/$\mu$mole). The presence of this marker permitted the later calculation of recovery of endogenous cAMP (about 50%) in fluke extracts. The mixture was then centrifuged at 12,000 × *g* for 20 min, and the resulting supernatant was neutralized with 5 N NaOH (about 10 $\mu$l). At this point the samples could be stored frozen before proceeding to a centrifugation at 17,000 × *g* for 20 min. To the supernatant was added a quantity of 0.25 M $ZnSO_4$ equal to 10% of the original volume, followed by enough saturated $Ba(OH)_2$ to neutralize the mixture. Samples were left in ice for 10 min and were then centrifuged at 8000 × *g* for 15 min. The supernatant fluid was treated again with $ZnSO_4$ and $Ba(OH)_2$ and centrifuged as before. For determination of endogenous cAMP recovery, 0.1 ml of the final supernatant fluid containing the marker was counted for $^{14}$C in 5 ml of Instagel (Packard Instrument Company). Counting was done in a Packard model 3320 liquid scintillation spectrometer. The remainder of the final supernatant fluid was used for measurement of cAMP concentration by the protein binding assay described above. The recovery of cAMP after precipitation by barium-zinc was 87%. The presence of fluke tissue extracts did not affect the protein binding assay.

To minimize the effect of individual differences between animals, at least six flukes were used in each experiment. Values for endogenous cAMP varied from one experiment to another, presumably because of the differing lengths of time (1–7 days) that the flukes had been maintained in the laboratory prior to use. Data presented here (with the exception of Table 1) are from representative experiments. Each experiment was repeated at least two times.

*Determination of 5-HT.* 5-HT was determined by the method of Snyder *et al.* (12). Since assays of 5-HT were performed on saline media and not on homogenates, the saline media were allowed to react directly with ninhydrin instead of being extracted with butanol-heptane.

*Materials.* The following compounds were purchased from Sigma Chemical Company: cAMP sodium salt; ATP sodium salt, Sigma grade; 5-hydroxydimethyltryptamine monooxalate hydrate; *N,N*-dimethyltryptamine; *d*-amphetamine sulfate; mescaline HCl; dopamine HCl; and GTP sodium salt, type III. Compounds purchased from Calbiochem were $N^6$,$O^2$-dibutyryl cAMP, A grade; 5-HT creatinine sulfate complex, B grade; 5-methoxytryptamine, A grade; tryptamine HCl, A grade; L-5-hydroxytryptophan, B grade; DL-octopamine HCl, B grade; histamine diphosphate, A grade; adenine, A grade; and adenosine, A grade. Norepinephrine (L-arterenol), L-epinephrine bitartrate, and glucagon were purchased from Winthrop, Mann, and Eli Lilly, respectively. The D-tartrate of LSD-25 was a product of Sandoz and was obtained from the National Institute of Mental Health. All other chemicals were reagent grade from various sources.

## RESULTS

*Activation of adenylate cyclase in fluke particles by 5-HT.* Experiments were carried out first to test the sensitivity of adenylate cyclase to 5-HT in different parts of the liver fluke. These studies were performed under conditions similar to those used in our original investigation with the whole organism (3). Under these conditions there was almost no enzyme activity in the absence of any activator. The caffeine added to the reaction mixture as a phosphodiesterase inhibitor effectively preserved the enhanced levels of cAMP which were produced in response to various effectors. Papaverine was found to be equally

effective as a phosphodiesterase inhibitor. In the presence of an activator the adenylate cyclase reaction was linear with time up to 16 min and with protein concentration over the range used (1.0–3.2 mg of protein per assay). Each experiment was designed to compare the activity of adenylate cyclase in heads, tails, and whole flukes from the same batch of organisms. Duplicate adenylate cyclase incubations gave values which differed by 5–10%. The results summarized in Fig. 1 show that particles prepared from fluke heads had higher adenylate cyclase activity in the presence of 5-HT than did particles from tails or whole flukes. Although a concentration of 5-HT as high as 1 mM was required for maximal activation, the enzyme was sensitive to a concentration as low as 0.1 μM. At this latter concentration the enzyme in particles from heads was activated to the extent of 3-fold, and at 10 μM 5-HT it was activated 19-fold.

Fluoride ion has been reported to activate almost every adenylate cyclase which has been discovered. The specific activity of fluke adenylate cyclase in response to fluoride appears to be among the highest known and yields a maximal level of 2.4–2.8 nmoles of cAMP per milligram per minute in particles from whole flukes. The optimal NaF concentration was 10 mM, and the smallest effective level was 1 mM. The half-maximal NaF concentration was about 3 mM. Addition of 0.1 mM 5-HT in the presence of NaF concentrations ranging from 1 to 2 mM caused a further (about 1.5-fold) increase in adenylate cyclase activity. However, in the presence of high NaF levels (5 mM), addition of 5-HT did not result in further activation of the enzyme. Similar results using combinations of NaF and 5-HT were previously reported (3). The specific activity of adenylate cyclase in particles from different parts of the organism showed smaller differences in the presence of saturating levels of NaF than with 5-HT. For example, in the presence of 10 mM NaF, adenylate cyclase activities were 3.9, 2.8, and 2.6 nmoles/mg·min in particles from heads, whole flukes, and tails, respectively. The greater difference in adenylate cyclase activity between the head and tail in the presence of 5-HT could be due, therefore, to a greater concentration of 5-HT receptors in the enzyme of the head.

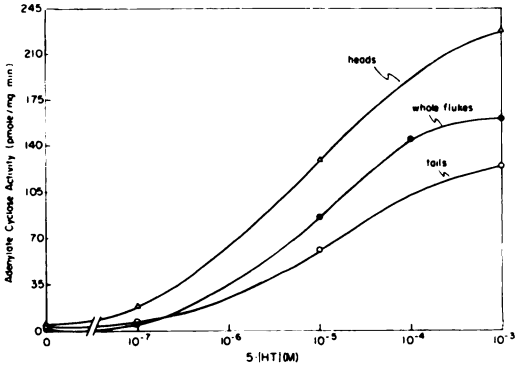


FIG. 1. *Activation of adenylate cyclase by 5-HT in particles from heads, tails, and whole flukes*

Suspensions of particles from at lest 20 heads, six tails, or six whole flukes were diluted 2-fold with 0.25 M sucrose and incubated in the adenylate cyclase reaction mixture with the indicated concentrations of 5-HT for 16 min. Boiled extracts of the reaction mixture were then assayed for cAMP. Adenylate cyclase activity was calculated as picomoles of cAMP accumulated per milligram of protein per minute. Other experimental details are given under METHODS.

*Effect of 5-HT on endogenous cAMP level.* Table 1 shows the increase in endogenous cAMP level in whole flukes, heads, or tails due to the presence of 1 mM 5-HT in the saline medium. These results clearly show that 5-HT caused a far greater increase in cAMP level in the head than in either the whole fluke or the tail. Furthermore, the degree of increase in cAMP level in the head was observed to be much larger relative to the tail or whole fluke than in the isolated particles.

Figure 2 demonstrates that both the cAMP level and the motility of the fluke are dependent on 5-HT concentration. The states of relative motility were measured subjectively and were clearly distinguished by the naked eye. An increase in motility was characterized in the tail by an increase in the rate and intensity of undulations and in the head by a more rapid, peristaltic movement. The process of severing, in itself, did not increase the motility of the head or tail. For heads, tails, or whole flukes, the increase in motor activity occurred within a few seconds after addition of 5-HT. The minimum concentra-

TABLE 1

*Effect of 5-HT on endogenous cAMP level in heads, tails, and whole flukes*

Twenty heads, six tails, or six whole flukes were incubated in saline medium with 1 mM 5-HT for 5 min. The flukes were then assayed for cAMP. Values for cAMP in heads or whole flukes are stated as means ± standard deviations. The difference between the means of control and 5-HT-treated whole flukes or heads was significant to the level of $\alpha = 0.01$ by the Wilcoxon ranked-pair differences test. Other experimental conditions were as described under METHODS.

| Fluke preparation | No. of experiments | Endogenous cAMP | | |
|---|---|---|---|---|
| | | Control | With 5-HT (1 mM) | Net increase |
| | | *pmoles/mg protein* | | |
| Whole | 6 | 19 ± 2 | 94 ± 56 | 75 ± 56 |
| Heads | 7 | 20 ± 6 | 760 ± 246 | 740 ± 246 |
| Tails | 1 | 17 | 201 | 184 |
| Tails | 1 | 12 | 43 | 31 |
| Tails | 1 | 14 | 51 | 37 |

tion of 5-HT required to cause an increase in either motility or cAMP level was 20 μM.

It was reasoned that the greater increase in the cAMP level of the severed head compared with the tail or whole fluke might be due to the higher ratio of cut surface area to total surface area in this part of the organism. This could result in a relatively greater permeability of the head to 5-HT. To investigate such a possibility, we compared the heads with tails which had been sliced into thin strips. These strips presented a cut surface area even larger than that of the severed head and, therefore, could have exaggerated the effect of permeability. The results showed that the sliced tails did have a greater increase in cAMP level in response to 5-HT than did unsliced tails. For example, incubation with 1 mM 5-HT for 5 min increased cAMP to 232 pmoles/mg of protein in sliced tails compared with 51 pmoles/mg of protein in unsliced tails. However, in the same experiment, the cAMP level in severed heads was elevated to 1004 pmoles/mg of protein in the presence of 5-HT. This represents a far greater increase than found with either sliced or unsliced tails. In the absence of 5-HT, slicing had no effect on cAMP levels. Thus the relatively large cut surface area of the severed head cannot entirely account for the greater sensitivity of the head to 5-HT.

Figure 3 depicts endogenous cAMP levels as a function of incubation time, using a fixed concentration of 5-HT (1 mM). Maximal accumulation of the cyclic nucleotide in the head, tail, or whole fluke was attained within 5–10 min, after which there was a rapid decrease to a plateau level slightly above control activity. The decrease in cAMP level was not accompanied by a reduction in fluke motility. We investigated the possibility that the decrease in cyclic AMP level was due to a depletion of 5-HT from the bathing medium. Determination of 5-HT concentration at intervals up to 2 hr, however, revealed no measurable change. Thus the decrease in the effect of 5-HT on the cAMP level does not appear to be due to inactivation of the amine by the fluke. Another possibility was that

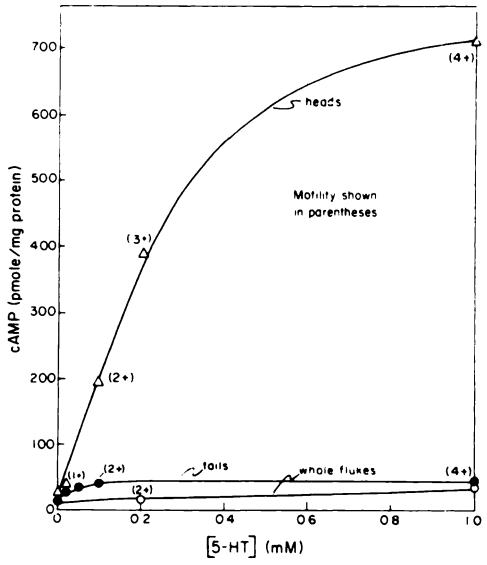


FIG. 2. *Endogenous cAMP level in heads, tails, and whole flukes as a function of 5-HT concentration*

In this representative experiment, 20 heads, six tails, or six whole flukes were incubated with the indicated concentrations of 5-HT for 5 min and then assayed for cAMP. Shown in parentheses is an estimation of relative motility, for which 0 indicates a resting state and 4+ indicates maximum motility. Intermediate states of motility are indicated by 1+, 2+, and 3+. Other experimental details are given under METHODS.

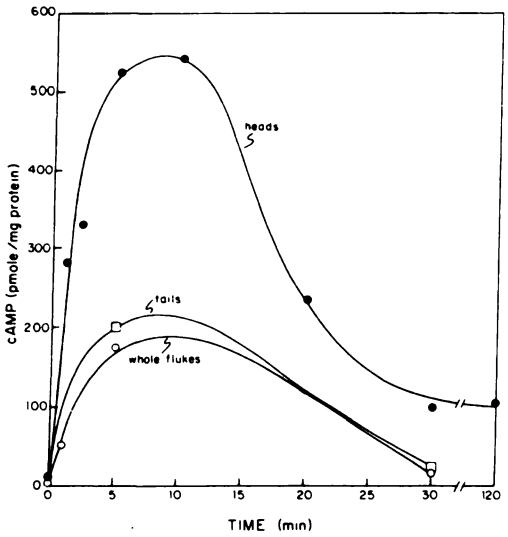


FIG. 3. *Time course for formation of endogenous cAMP in heads, tails, and whole flukes*

In this representative experiment, 20 heads, six tails, or six whole flukes were incubated with 1 mM 5-HT for the indicated times and then assayed for cAMP. Other experimental conditions were as described under METHODS.

cAMP was being excreted from the organism into the incubation medium. We were unable, however, to detect any cAMP in the medium at intervals up to 2 hr.

*Comparison of 5-HT with action of other effectors on fluke motility and adenylate cyclase activity.* Table 2 presents a comparison of the effects of 5-HT with selected analogues and NaF on motility, endogenous cAMP in fluke heads, and adenylate cyclase activity in particles from whole flukes. 5-HT caused by far the greatest increase in endogenous cAMP level and, except for NaF, caused the highest activation of adenylate cyclase in fluke particles. As in the case of 5-HT, the analogues produced a motility increase within a few seconds after addition. The closest structural analogue to 5-HT tested was 5-methoxytryptamine. This indoleamine caused a 4-fold increase in motility but a much diminished effect on both endogenous cAMP level and adenylate cyclase activity in particles. Four other 5-HT analogues—tryptamine, 5-hydroxydimethyltryptamine, 5-

TABLE 2

*Effects of various agents on motility, endogenous cAMP level, and adenylate cyclase activity in particles*

In this representative experiment, motility of whole flukes was estimated as described in Fig. 3. The endogenous cAMP concentration (in picomoles per milligram of protein) was determined in 20 fluke heads which had been incubated with the test substance for 5–10 min or 30 min. For the determination of both motility and endogenous cAMP level, the concentration of test substance was 1 mM except for LSD and NaF, which were tested at 0.1 mM and 10 mM, respectively. Since values for endogenous cAMP were obtained on different batches of flukes, cAMP levels in the control ranged from 2 to 32 pmoles/mg protein. Because of this variation, each value is given as a multiple of its control in the same experiment. This permits a comparison to be made of the effects of different agents. Adenylate cyclase activity was determined on suspensions of particles from at least 20 whole flukes. The concentration of test substance in the adenylate cyclase assay mixture was 0.1 mM except for NaF, which was tested at 5 mM. The assay mixture was incubated for 16 min. The adenylate cyclase activity experiments were done on the same fluke preparation, and the values are given as multiples of the control, which was 1.6 pmoles of cAMP per milligram of protein per minute. Other experimental conditions were as described under METHODS.

| Addition | Estimate of motility | Endogenous cAMP in heads | | Adenylate cyclase activity in particles |
|---|---|---|---|---|
| | | 5–10 min | 30 min | |
| | | × *control* | | × *control* |
| None | 0 | 1.0 | 1.0 | 1.0 |
| 5-Hydroxytryptamine | 4+ | 37.2 | 12.7 | 38.8 |
| 5-Methoxytryptamine | 4+ | 6.0 | 5.0 | 20.1 |
| Tryptamine | 4+ | 2.0 | | 11.8 |
| 5-Hydroxydimethyltryptamine | 3+ | 1.2 | 2.0 | 9.0 |
| 5-Hydroxytryptophan | 2+ | 1.2 | 2.0 | 3.0 |
| Dimethyltryptamine | 1+ | 1.1 | 1.0 | 1.6 |
| LSD | 4+ | 1.0 | 1.0 | 5.2 |
| Amphetamine | 4+ | 1.0 | | 2.3 |
| NaF | 0 | 1.0 | 2.0 | 1518.0 |

hydroxytryptophan, and dimethyltryptamine—produced decreasing responses in both the stimulation of motility and the activation of adenylate cyclase. This effect corresponds to a decrease in structural similarity of the analogues to 5-HT. For example, 5-HT has both a free amino and a 5-hydroxyl group, whereas dimethyltryptamine, which was ineffective, has neither of these moieties. Analogues which were intermediate in their effect on motility and adenylate cyclase activity lack either the free amino or the 5-hydroxyl group. These results reveal the importance of these two moieties in the effect of 5-HT on fluke adenylate cyclase. It should be noted that the concentration chosen for each effector was that which had a maximal effect on motility increase. Correlations of motility and cAMP levels at other concentrations of analogues must await future studies.

LSD (which contains both indole and diethylamide groups) and amphetamine (a phenylethylamine) both strongly stimulated motility. The minimum concentration of LSD required to increase motility was 1 nM. This value is over three orders of magnitude lower than that required by 5-HT and the other analogues. Furthermore, maximal motility was caused by 1 μM LSD, compared with 1 mM for the other compounds tested. This demonstrates that LSD was the most potent stimulator of fluke motility among those analogues shown in Table 2. However, neither LSD (from 0.1 nM to 1 mM) nor amphetamine (1 mM) had any measurable effect on endogenous cAMP level. Since it was possible that LSD caused an increase in cAMP level in the head prior to 5 min of incubation, we measured cAMP at intervals from 15 sec to 5 min after addition of LSD. We were unable to detect any increase in endogenous cAMP during this period. As previously reported (3), LSD slightly activated adenylate cyclase in particles. A comparison of LSD with 5-HT as an activator of adenylate cyclase in particles is summarized in Fig. 4. Maximal activity in the presence of LSD was only 14 pmoles/mg·min, compared with 71 pmoles/mg·min with 5-HT. Amphetamine had no effect on adenylate cyclase activity in particles. The results for LSD and amphetamine show that their ability to stimulate rhythmical movement in the fluke had little correlation with their effect on adenylate cyclase activity. This is in contrast to the results with the indoleamines, which displayed a high correlation between their effects on motility and adenylate cyclase activity.

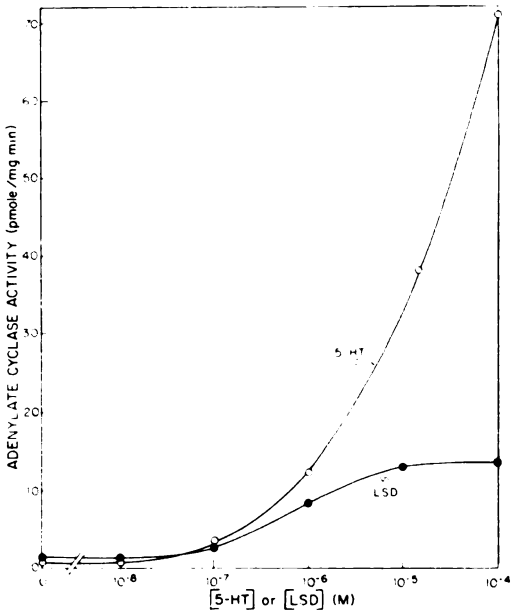


FIG. 4. *Comparison of 5-HT and LSD as activators of adenylate cyclase in particles from whole flukes*

Suspensions of particles from at least six whole flukes were incubated in the adenylate cyclase reaction mixture for 16 min with the indicated concentrations of 5-HT or LSD. Boiled extracts were then assayed for cAMP. Adenylate cyclase activity was calculated in picomoles of cAMP accumulated per milligram of protein per minute. Other experimental details are given under METHODS.

Fluoride ions have no apparent structural similarity to any of the 5-HT analogues but produced the greatest activation of adenylate cyclase in particles. Table 2 also demonstrates that incubation of fluke heads for 30 min with 10 mM NaF produced a 2-fold increase in cAMP level. In experiments not shown here, tails did not demonstrate a similar effect. Fluoride had no effect on fluke motility.

*Antagonism by LSD of 5-HT effect on adenylate cyclase.* While LSD and 5-HT both stimulated rhythmical movement in the liver fluke, previous pharmacological

experiments on different tissues showed that LSD could antagonize some of the effects of 5-HT. For example, LSD was shown to antagonize the effect of 5-HT on sensitized rat uterus (13). We examined the effect of LSD on adenylate cyclase activation by 5-HT. The results revealed that in the presence of 0.1 mM 5-HT the adenylate cyclase activity in the fluke particles was inhibited by as little as 0.5 $\mu$M LSD. A maximal inhibition of about 50% was caused by 0.1 mM LSD. Still more pronounced was the antagonism by LSD of the 5-HT-stimulated endogenous cAMP levels in intact organisms (Fig. 5). With increasing concentrations of LSD, the 5-HT activation was diminished until it was completely blocked at about 0.1 mM LSD. The concentration of LSD that caused half-inhibition of the 5-HT effect was 5 nM. This indicates that LSD has a very high affinity for its site of action. Reversing the order of addition of 5-HT and LSD with either the particles or intact flukes yielded nearly identical curves. The kinetics of LSD antagonism appears to be complex.

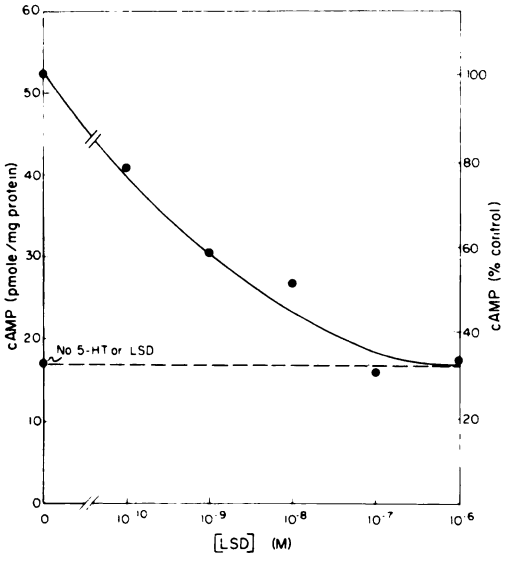


FIG. 5. *LSD antagonism of endogenous cAMP elevated by 5-HT in whole flukes*

In this representative experiment, six whole flukes were incubated with 1 mM 5-HT for 1 min. The indicated concentration of LSD was then added, and after an additional 5-min incubation the flukes were assayed for cAMP. The control was a sample incubated with 5-HT alone for 6 min. Other experimental details are given under METHODS.

Lineweaver-Burk plots, which were generated by testing the activity of the particles with various 5-HT levels in the presence of fixed increments of LSD, did not yield straight lines, suggesting that the kinetics is not first-order. As shown above, LSD at low concentrations activates adenylate cyclase. The possibility existed that LSD antagonism of adenylate cyclase activation by 5-HT was due to an increase in the rate of destruction of cAMP by phosphodiesterase. We found, however, that LSD had no direct effect on fluke phosphodiesterase activity.

Experiments were designed to test the reversibility of the effect of 5-HT and LSD on fluke motility. Intact flukes which had been incubated for 5 min in the presence of 1 mM 5-HT or 10 $\mu$M LSD were thoroughly washed and transferred to fresh saline medium. 5-HT-treated flukes returned to a resting level of motility within 30 min, whereas flukes treated with LSD required 2 hr to return to a resting state. The flukes were continually washed (at least 10 times) over these time periods. These results suggest that the affinity of LSD for receptors in the flukes is greater than that of 5-HT, and are consistent with the finding of a low half-inhibition constant for LSD.

*Compounds which had no effect on fluke motility or adenylate cyclase activity.* We tested the following substances and found that they had no effect on fluke motility, endogenous cAMP level, or adenylate cyclase activity in particles: adenine, adenosine, epinephrine, histamine, and tryptophan. Furthermore, dopamine, glucagon, GTP, and norepinephrine had no effect on motility or adenylate cyclase activity in particles and were not tested for an effect on endogenous cAMP. cAMP itself or dibutyryl cAMP had no effect on fluke motility. For motility and endogenous cAMP, the tested concentration of each substance was 1 mM except for glucagon, which was tested at 100 $\mu$M. For adenylate cyclase activity in particles, the tested concentration was 100 $\mu$M except for glucagon, which was tested at 10 $\mu$M.

## DISCUSSION

In an investigation into the control of adenylate cyclase, it is important to re-

late hormonal activation of the enzyme in isolated particles with an increase in cAMP level in the intact tissue following exposure to the hormone. The present experiments demonstrate that 5-HT both activates liver fluke adenylate cyclase in particles and causes a marked accumulation of endogenous cAMP, particularly in the anterior end of the organism. The increase in endogenous cAMP levels after exposure of the fluke to 5-HT was rapid, reaching a maximum after only 5 min. Thus 5-HT appears to penetrate readily into the intact organism in spite of the existence of a cuticle surrounding the entire body. The high levels of cAMP were not maintained for a long time but gradually decreased over a period of 30 min. It is probable that there is a regulatory mechanism associated with the adenylate cyclase system that is responsible for such a decrease. Ho and Sutherland (14) observed a similar effect with epinephrine on rat epididymal adenylate cyclase and attributed it to the presence of an inhibitor which acts to decrease the sensitivity of the enzyme to the hormone. Another possibility could be an increase in the activity of phosphodiesterase in the fluke.

Anatomical studies of the trematode indicate the presence of a circumpharyngeal nerve ring in the anterior end of the organism. Heads isolated in the present investigation included that part of the neuronal system. In the presence of 5-HT, the specific activity of adenylate cyclase was approximately twice as much in particles isolated from heads compared with tails. Furthermore, 5-HT caused a much greater increase in the endogenous cAMP level in the heads than it did in the tails. The greater sensitivity of adenylate cyclase in the head to the effect of 5-HT could be due to the nature or number of 5-HT receptors in this part of the fluke. The localization of these receptors may be related to the higher concentration of neuronal tissue in the head. The posterior part of the organism, while containing some neuronal tissues, is composed mainly of genitalia and digestive tract. It has been proposed that 5-HT or a related alkylindoleamine plays a regulatory role in many invertebrates (15). Subsequent to our early findings that 5-HT activates adenylate cyclase in the liver fluke (3) there have been many reports showing 5-HT activation of adenylate cyclase from other invertebrate species. These include adult schistosome (16), abdominal ganglia of *Aplysia* (17), thoracic ganglia of cockroach (18), and salivary gland of blowfly (19). The results tend to support the hypothesis that cAMP acts as the second messenger for 5-HT action in these invertebrates. A role for cAMP in mediating the effect of 5-HT on glycogenolysis and glycolysis in the fluke has already been suggested. On the other hand, the role of cAMP in the regulation of neuromuscular activity is still obscure. Results of the present investigation suggest no direct relationship between the elevation of cAMP levels and the stimulation of rhythmical movement. For example, amphetamine, which stimulated fluke motility, showed no effect on cAMP levels either in particles or in the intact organism. LSD, the most potent stimulator of muscular activity, had a slight activating effect on adenylate cyclase in particles but antagonized 5-HT activation of the enzyme both in particles and in intact organisms. We previously reported that both 5-HT and cAMP can increase glycolysis in cell-free extracts of the fluke. It is possible, therefore, that 5-HT simultaneously affects both motility and carbohydrate metabolism but that only the latter process is necessarily mediated by cAMP.

The results with LSD deserve special analysis, since at low concentrations this compound activated adenylate cyclase but at higher concentrations it antagonized the effect of 5-HT on the enzyme. A dual effect of LSD on adenylate cyclase was also reported by Nathanson and Greengard (18) on the enzyme from cockroach thoracic ganglia. The cockroach enzyme appears to be different from the fluke enzyme, in that antagonism by LSD of the 5-HT effect occurred at concentrations of LSD lower than those necessary for enzyme activation. Furthermore, the specificity of the enzyme from the two sources appears to be different. While activation of the fluke enzyme was highest with indoleamines, the cockroach enzyme was more strongly activated by octopamine, norepinephrine,

and dopamine than it was by 5-HT. The effect of LSD on cAMP accumulation in tissues does not appear to be restricted to invertebrates. Palmer and Burks (20) reported antagonism by LSD on the epinephrine-stimulated increase of endogenous cAMP in rat brain slices. It is possible that simple binding of either 5-HT or LSD may be sufficient for neuromuscular excitation but that a degree of reversibility of the effector-receptor complex is required for activation of adenylate cyclase. Our results indicate that the affinity of LSD may be too high to permit sufficient reversibility. This idea is supported both by the finding of an extremely low half-inhibition constant for LSD (5 nM) in the intact fluke and by the observation that recovery of the fluke after stimulation with LSD took a much longer time than recovery following treatment with 5-HT. These concepts agree with the suggestion by Berridge and Prince (21) that LSD and 5-HT interact with the same tryptaminergic receptor, but that the action of LSD is prolonged because it fails to disengage properly from the active site. An alternative possibility is that the LSD-receptor complex is inherently less efficient than the 5-HT–receptor complex at activating adenylate cyclase. This could be due to a lack of appropriate interactions between the LSD molecule and the receptor.

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