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

II. Activation of Protein Kinase by 5-Hydroxytryptamine

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SUMMARY

GENTLEMAN, SUSAN, ABRAHAMS, SANDERS, L. & MANSOUR, TAG, E. (1976) Adenosine cyclic 3',5'-monophosphate in the liver fluke, *Fasciola hepatica*. II. Activation of protein kinase by 5-hydroxytryptamine. *Mol. Pharmacol.*, 12, 59-68.

Adenosine cyclic 3',5'-monophosphate (cAMP)-dependent protein kinase activity has been found in both the 27,000 × *g* particulate and supernatant fractions of homogenates from the liver fluke, *Fasciola hepatica*. The enzyme activity was 2–5 times greater in the anterior end ("head") of the fluke than in the posterior end. Half-maximal activation of the protein kinase in both fractions was obtained at 0.1–0.4 μM cAMP. When the enzyme was assayed in the presence of cyclic nucleotide, the apparent K_m for protamine was 0.1 mg/ml and the apparent K_m for MgATP was 20–50 μM in the two homogenate fractions. Incubation of fluke heads for 5 min with 1 mM 5-hydroxytryptamine resulted in an increase in protein kinase activity of both homogenate fractions when assayed in the absence of added cAMP. The protein kinase activity assayed in the presence of 5 μM cAMP was increased in the particulate fraction and decreased in the supernatant fraction. The degree of activation of the enzyme by the cyclic nucleotide was markedly reduced in both homogenate fractions from heads that had been incubated with 5-hydroxytryptamine. The time course of accumulation of endogenous cAMP correlated with activation of protein kinase, except during the first minute of incubation with 5-hydroxytryptamine. The degree of activation of protein kinase by added cAMP was inversely correlated with endogenous cyclic nucleotide during incubation with 5-hydroxytryptamine. Incubation of fluke heads with both 5-hydroxytryptamine and D-lysergic acid diethylamide together reduced the activation of protein kinase by 5-hydroxytryptamine. Incubation of fluke heads with only D-lysergic acid diethylamide had no effect on protein kinase activity. cAMP increased phosphorylation of fluke protein in both fractions of the homogenate. This phosphorylation was less sensitive to activation by the cyclic nucleotide when the fluke heads had been incubated with 5-hydroxytryptamine. Thus 5-hydroxytryptamine can stimulate protein kinase activity toward endogenous proteins. The results indicate that the physiological effects of 5-hydroxytryptamine are mediated by cAMP activation of protein kinase.

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INTRODUCTION

Hormonal regulation of cellular functions which are mediated by cAMP[3] is often achieved by activation of protein kinases, which phosphorylate specific proteins (1). The classical example of this type of hormonal regulation is the activation of glycogen phosphorylase by epinephrine. The hormonal activation of cAMP-dependent protein kinase was reported recently in several mammalian tissues, both *in vitro* (2, 3) and *in vivo* (4, 5). It has been suggested that the hormonal effect is not only to activate protein kinase but also to change the distribution of the enzyme activity in the cell (2, 5). Subcellular localization of protein kinase in membrane fractions has been implicated in the regulation of synaptic transmission, ion transport, and membrane permeability in several tissues (6–9). Thus the distribution of cAMP-dependent protein kinase in the particulate and soluble cell fractions may be a mechanism for mediating the diverse effects of a hormone on the cell.

Previous work in our laboratory showed that 5-HT was a potent stimulator of motility (10, 11) and carbohydrate metabolism (12, 13) in the liver fluke, *Fasciola hepatica* (Platyhelminthes: Trematoda). 5-HT was also shown to activate adenylate cyclase and to increase accumulation of endogenous cAMP in liver flukes (14, 15). Thus, in these organisms, 5-HT appears to have a role similar to that of epinephrine in mammals. In this paper we have investigated the effect of 5-HT on protein kinase activity in the liver fluke. We also studied the effect of LSD, a drug which stimulates motility (11) and metabolism (13) in the fluke but which antagonizes 5-HT activation of adenylate cyclase (15).

METHODS

Fasciola hepatica were collected from fresh beef livers and maintained in the laboratory as previously described (16). Before being used in an experiment, the flukes were rinsed in warm (37°) saline solution (16). Unless otherwise indicated, the anterior portion of the fluke ("head") was then severed from the posterior portion ("tail") just below the posterior sucker. The heads, tails, or intact flukes were placed in fresh warm saline solution and used within 30 min. Protein kinase activity in fluke heads which had been preincubated for up to 30 min did not change significantly. The experiment was initiated by the addition of 1 mM 5-HT and/or 10 μM LSD to the incubation solution. After 5 min the tissues were removed from the solution, drained, and frozen with aluminum clamps chilled in Dry Ice. The tissues were homogenized by hand (10 strokes) in a conical glass homogenizer with 20 volumes of cold 50 mM glycylglycine (pH 7.5) containing 10 mM NaF and 5 mM theophylline. Tissue from two animals was used for each preparation. The homogenate was centrifuged at 27,000 × *g* for 30 min. The supernatant fraction was removed, and the pellet (particulate fraction) was resuspended to the initial volume with cold homogenizing solution. The protein concentration of each fraction was determined by the method of Lowry *et al.* (17).

Protein kinase activity of the supernatant and particulate fractions was assayed by the method of Miyamoto *et al.* (18). The standard 0.2-ml reaction volume contained 50 mM sodium glycerol phosphate (pH 6.5), 10 mM magnesium acetate, 10 mM NaF, 2 mM theophylline, 0.3 mM EDTA, 40 μg of protamine chloride, 2.5 μM [γ-^{32}P]ATP (specific activity, 0.5–5.0 mCi/μmole), and 25 μl of fluke supernatant or particulate fraction (15–25 μg of protein). Each fraction was assayed with and without 5 μM cAMP. After 5 min at 25°, the reaction was stopped by the addition of 1.8 ml of cold 10 mM EDTA containing 1 mM unlabeled ATP. To precipitate the protein, 2 ml of cold 20% (w/v) trichloroacetic acid were added. After 15–30 min the precipitate was collected on a Millipore filter (HAWP 02400) and washed three times with 5 ml of 5% trichloroacetic acid. The filters were air-dried and counted in toluene-based scintillation fluid. Protamine was quantitatively precipitated by this procedure over the concentrations used. Two types of blanks were used for each assay. The zero-

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

time blank was obtained by adding the cold EDTA-ATP solution before the addition of the fluke preparation and contained less than 0.001% of the total counts in the assay solution. The second blank was incubated without protamine and permitted the determination of phosphate incorporation into the fluke preparation. The value of this blank was 25–30% of that obtained in the presence of protamine. The net ^{32}P bound to protamine was calculated by subtraction of this latter blank. A unit of protein kinase activity is defined as 1 pmole of phosphate transferred from ATP to protamine per minute. Under the assay conditions given, the reaction was linear with time up to 10 min and linear with fluke protein concentration up to 125 μg/ml of reaction volume. Duplicates of the protein kinase assay differed by no more than 5%. The specific activity of protein kinase was dependent on the length of time the flukes had been maintained in the laboratory. Heads of freshly collected flukes had a specific activity of 30–40 units/mg of protein (assayed in the presence of 5 μM cAMP). After 2 weeks in the laboratory, the specific activity was 3–4 units/mg. However, the responses of protein kinase to 5-HT and LSD were observed in all flukes regardless of the length of time they had been kept in the laboratory.

The concentration of cAMP in fluke tissues and in the homogenate fractions was determined as previously described (15).

A modification of the method of Kennell (19) was used to determine the distribution of the ^{32}P label among phospholipid, polynucleotide, and protein in the trichloroacetic acid precipitates. Fluke homogenate fractions were precipitated with 10% trichloroacetic acid after incubation in the protein kinase assay solution without protamine. The precipitates were washed three times with cold 5% trichloroacetic acid and once with cold 70% ethanol and collected on Nucleopore filters (N 300 CPR 02500). The filters were then washed twice with warm (40°) 70% ethanol, twice with 70% ethanol–diethyl ether (1:1), and once with diethyl ether to extract phospholipids. The washes were collected, evaporated, and counted in Instagel. The filters were put into test tubes with 2 ml of 5% trichloroacetic acid and heated in a water bath at 80% for 30 min to solubilize polynucleotides. The tubes were then placed in an ice bath for 30 min. The contents of the test tubes, including the filters, were collected on fresh Nucleopore filters and washed three times with cold 5% trichloroacetic acid and once with cold 70% ethanol. The washed were pooled, and the tricholoracetic acid was removed with three ether rinses. Aliquots were taken and counted in Instagel. The filters were incubated in 1 ml of 1 N NaOH for 30 min at 37° to solubilize the protein. Aliquots were taken for counting in Instagel. Protein, as determined by the method of Lowry *et al.* (17), was present exclusively in the material solubilized from the filters by NaOH. The A_{260}:A_{280} ratio of the material solubilized with hot trichloroacetic acid was 1.8–2.0, indicating the presence of nucleotides.

[γ-^{32}P]ATP was prepared by the method of Post and Sen (20). Disodium ATP, cAMP, and protamine chloride were obtained from Sigma Chemical Company. 5-HT (B grade, creatinine salt) was obtained from Calbiochem, and theophylline, from Merck. The D-tartrate of LSD-25 was a product of Sandoz and was obtained from the National Institute of Mental Health.

RESULTS

Effect of substrates and cAMP concentrations on protein kinase activity. Figure 1 shows protein kinase activity of the particulate and supernatant fractions with respect to protamine concentration in the presence of cAMP. The apparent K_m of the enzyme for protamine was 0.1 mg/ml in both fractions. The V_{max} was 91 units/mg in the particulate fraction and 80 units/mg in the supernatant fraction. In the absence of cAMP, maximal activity in both fractions was obtained at 0.1 mg/ml of protamine and was only 20% of that obtained in the presence of cAMP. Because of this low activity, it was not possible to obtain reproducible kinetics at nonsaturating protamine concentrations. The protein kinase activity of the particulate and supernatant fractions of fluke head homogenates with respect to MgATP at a saturating protamine concentration is shown in Fig. 2. In

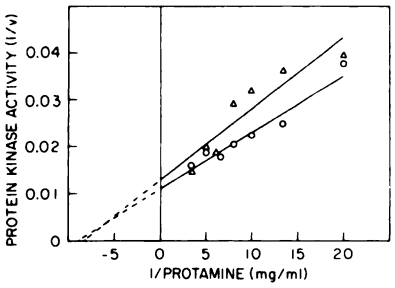

FIG. 1. *Activity of protein kinase in homogenate fractions from fluke heads as a function of protamine concentration*

Fluke heads were homogenized and centrifuged as described in METHODS. Protein kinase activity of each fraction was assayed with 5 μM cAMP at various protamine concentrations. Slopes and intercepts of the lines were determined by the method of least squares. The correlation coefficient r was +0.93 or greater. ○, particulate fraction; △, supernatant fraction.

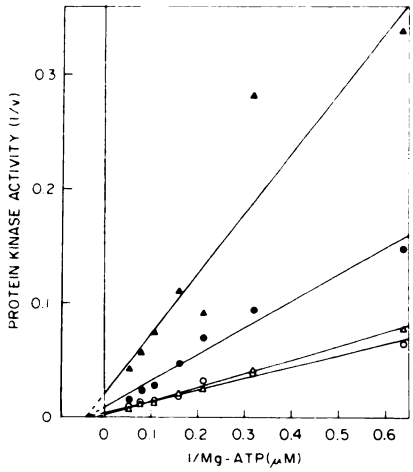

FIG. 2. *Protein kinase activity in homogenate fractions from fluke heads as a function of MgATP concentration*

Homogenate fractions of fluke heads were prepared and assayed for protein kinase activity as described in METHODS, except that the MgATP concentration was varied. Slopes and intercepts of the lines were determined by the method of least squares. The correlation coefficient r was +0.95 or greater. ○ and ●, particulate fraction; △ and ▲, supernatant fraction; ● and ▲, assayed without cAMP; ○ and △, assayed with cAMP.

the absence of cAMP, the apparent K_m for MgATP of both fractions was about 23 μM. The V_{max} values of the supernatant and particulate fractions were 45 and 100 units/mg, respectively. In the presence of cAMP, the apparent K_m for MgATP was 20 μM in the particulate fraction and 45 μM in the supernatant fraction. The V_{max} values were 200 and 400 units/mg in the particulate and supernatant fractions, respectively. Thus the data from the representative experiments shown in Figs. 1 and 2 indicate that the kinetic properties of protein kinase from the two fractions are similar. This similarity was observed in both the presence and absence of cAMP. In both the particulate and supernatant fractions, 0.1–0.4 μM cAMP caused 50% activation of the enzyme. Maximum activation of the protein kinase in both fractions was obtained in the presence of 1–3 μM cAMP. Thus there does not appear to be any significant difference in kinetic properties of protein kinase between the two fractions with respect to cAMP.

Distribution of protein kinase activity in liver fluke. Table 1 summarizes a representative experiment showing the distribution of protein kinase activity between the particulate and supernatant fractions of homogenates from heads, tails, and whole flukes. The specific activity of homogenate fractions from heads was 2–5 times greater than that of fractions from tails or from whole flukes. It was considered possible that this difference between the heads and tails was due to a greater phosphodiesterase activity in the tails during the protein kinase assay. However, determination of the cAMP concentration at the end of the protein kinase assay showed that there was no loss of cAMP. The distribution of protein kinase activity between the fractions of fluke head homogenates was about 60% in the supernatant and 40% in the particulate fraction. The protein kinase in both fractions was activated by cAMP. In the particulate fraction the activation by cAMP was 4.3-fold, and in the supernatant it was 5.5-fold (means of 10 experiments).

Although incubation of fluke heads with 5-HT strongly activated protein kinase, incubation of the tails had no effect on protein kinase activity (Table 1). These results correlate well with the localization of 5-HT-activated adenylate cyclase in the head of the fluke (15). In all the experiments described below, only the the isolated heads of flukes were used.

5-HT activation of protein kinase in fluke heads. 5-HT has been shown to increase endogenous cAMP levels in the fluke (15). We hypothesized that such an increase in cAMP would lead to activation of protein kinase in the intact cell. Table 2 shows a statistical analysis of the changes in protein kinase activity in the particulate and supernatant fractions from fluke heads incubated in 1 mM 5-HT. Although there was an increase in the protein kinase activity of both fractions assayed without added cAMP, the increase in supernatant activity was not statistically significant. This activated protein kinase would be expected to be less sensitive to further activation by cAMP added to the assay solution. Thus the degree of activation by cAMP in the particulate fraction decreased from 4.3-fold in the absence of 5-HT to 1.6-fold with 5-HT. In the supernatant fraction the degree of activation decreased from 5.5-fold to 2.5-fold. Incubation of the fluke heads with 5-HT also appeared to affect the distribution of protein kinase activity between the particulate and supernatant fractions when assayed in the presence of cAMP. As shown in Table 2, incubation with 5-HT caused a 2-fold increase in protein kinase activity in the particulate fraction and a 33% decrease in the activity in the supernatant fraction (see also Table 1). These data may reflect a translocation of protein kinase

TABLE 1

Distribution of protein kinase activity in liver flukes

Heads, tails, and whole flukes were incubated for 5 min in the presence and absence of 5-HT. The tissue was then homogenized and centrifuged as described in METHODS. Protein kinase activity in the 27,000 × *g* particulate and supernatant fractions was assayed with and without 5 μM cAMP.

Body part	Addition to incubation solution	Protein kinase activity			
		Particulate fraction		Supernatant fraction	
		Without cAMP	With cAMP	Without cAMP	With cAMP
		units/mg		*units/mg*	
Heads	None	5.0	23.2	4.2	28.4
Heads	1 mM 5-HT	17.2	29.3	5.4	10.6
Tails	None	1.6	10.9	0.7	5.8
Tails	1 mM 5-HT	2.3	7.0	0.5	3.8
Whole flukes	None	1.3	9.2	0.9	6.7
Whole flukes	1 mM 5-HT	2.4	9.8	0.8	5.0

TABLE 2

Effect of incubation with 5-HT on protein kinase activity in fluke heads

Fluke heads were incubated in the presence and absence of 5-HT. The tissues were prepared and assayed for protein kinase as described in Table 1. Values given are means ± standard errors. The degree of activation by cAMP is the ratio of protein kinase activity obtained in the presence of 5 μM cAMP to the activity obtained without added cAMP.

Addition to incubation solution	No. of experiments	Particulate fraction			Supernatant fraction		
		Protein kinase activity		Degree of activation	Protein kinase activity		Degree of activation
		Without cAMP	With cAMP		Without cAMP	With cAMP	
		units/mg			*units/mg*		
None	11	3.5 ± 1.0	11.8 ± 3.6	4.3 ± 0.9	3.2 ± 0.8	14.2 ± 2.2	5.5 ± 0.7
1 mM 5-HT	10	12.3 ± 3.9[a]	19.0 ± 3.2[a]	1.6 ± 0.1[a]	4.5 ± 1.0	10.2 ± 1.2[a]	2.5 ± 0.5[a]

[a] Significantly different from means of untreated fluke heads ($p < 0.01$ by the paired-value *t*-test, one-tailed).

activity from the supernatant to the particulate fraction. However, the total increase in the specific activity of the particulate fraction was greater than can be accounted for by the decrease in the activity of the supernatant fraction. These changes in the specific activity represent changes in activity per original wet weight, since the different fractions were diluted to the same extent. The above effects of 5-HT on protein kinase activity in fluke heads appear to have been mediated by endogenous cAMP.

We reasoned that these effects might be mimicked by homogenization of control fluke heads in a concentration of cAMP equal to that achieved within the head in response to 5-HT. Assuming a uniform intracellular distribution of the nucleotide, we calculated a minimal value of 5 μM cAMP. As shown in Table 3, this concentration of cAMP, when added to the homogenizing solution, caused an increase in protein kinase activity, a reduction in the degree of activation by cAMP in the assay, and a redistribution of enzyme activity between the two fractions. These effects occurred to about the same extent as they did when the fluke was incubated in 5-HT. We also determined that the concentration of cAMP carried over into the crude homogenate of fluke heads which had been incubated in 5-HT was about 0.1 μM. When control heads were homogenized in this latter concentration of cAMP, there was no effect on protein kinase activity. These results support the hypothesis that the effects of 5-HT on protein kinase activity occur before homogenization as a result of high concentrations of cAMP *in vivo*. A remaining possibility was that the effects of 5-HT on protein kinase activity were due to a carryover of cAMP into the assay solution. Measurement of the cAMP carried over showed that its concentration was less than 15 nM, a value too low to account for the observed effects. It may also be seen in Table 3 that the presence of theophylline in the homogenizing solution preserved the effects of 5-HT on protein kinase activity, since the effects were reduced when this phosphodiesterase inhibitor was omitted.

Relationship between protein kinase activity and time of incubation with 5-HT. In these experiments fluke heads were incubated with 5-HT for several time intervals. At the end of each interval the endogenous cAMP level and protein kinase activity were determined.

Figure 3A summarizes a representative experiment showing changes in protein kinase activity in the supernatant fraction of head homogenates. When assayed in the absence of cAMP, the enzyme activity increased 2-fold during the first 5 min of incubation with 5-HT. After incubation times longer than 5 min this protein kinase activity was reduced. These changes in enzyme activity corresponded to the changes in endogenous cAMP level. When assayed

TABLE 3

Effect of homogenization of fluke heads with cAMP and theophylline on protein kinase activity

Fluke heads were incubated for 5 min in the presence and absence of 5-HT. They were then homogenized in the standard solution (50 mM glycylglycine, pH 7.5, containing 10 mM NaF and 5 mM theophylline) with and without 5 μM cAMP, or in the standard solution without theophylline. The homogenates were centrifuged and assayed for protein kinase activity as described in Table 1.

Addition to fluke incubation solution	Homogenizing solution	Protein kinase activity			
		Particulate		Supernatant	
		Without cAMP	With cAMP	Without cAMP	With cAMP
		units/mg		*units/mg*	
None	Standard	6.3	23.8	10.8	55.6
1 mM 5-HT	Standard	34.2	55.6	16.1	20.2
None	5 μM cAMP added	35.6	44.4	20.0	20.4
1 mM 5-HT	Theophylline omitted	13.7	39.7	8.1	37.8

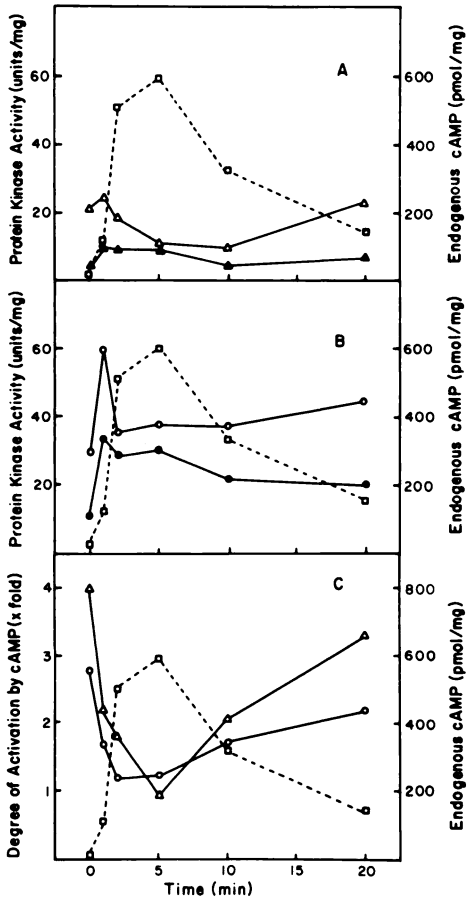

FIG. 3. *Protein kinase acitivity and endogenous cAMP level in fluke heads as a function of incubation time in the presence of 5-HT*

Fluke heads were incubated for varying lengths of time in the presence of 1 mM 5-HT. At each time interval, homogenate fractions of heads were prepared and assayed for protein kinase activity and endogenous cAMP as described in METHODS.

A. Protein kinase activity of the supernatant fraction. ▲, activity assayed without cAMP; △, activity assayed with cAMP; □, endogenous cAMP concentration.

B. Protein kinase activity of the particulate fraction. ●, activity assayed without cAMP; ○, activity assayed with cAMP; □, endogenous cAMP concentration.

C. Degree of activation of protein kinase by 5 μM cAMP. The correlation coefficient r between degree of activation and endogenous cAMP level is −0.89 for each homogenate fraction. ○, particulate fraction; △, supernatant fraction; □, endogenous cAMP concentration.

in the presence of cAMP, the supernatant activity was decreased to 50% of the initial activity after 5 min of incubation with 5-HT. At intervals longer than 5 min the protein kinase activity increased, until it returned to the initial level by 20 min of incubation. These changes in enzyme activity assayed in the presence of cAMP correlated inversely with the changes in endogenous cAMP level.

Figure 3B shows the changes in protein kinase activity in the particulate fraction of fluke head homogenates. When assayed in the absence of cAMP, the enzyme activity increased 3-fold during the first minute of incubation with 5-HT and then decreased slightly after 2 min. After incubation for more than 5 min the enzyme activity was decreased to only twice the initial value. These changes in protein kinase activity corresponded to the changes in endogenous cAMP levels. Protein kinase activity assayed in the presence of cAMP was decreased 2-fold at 1 min of incubation with 5-HT. After incubation for more than 1 min, enzyme activity in the presence of cAMP was decreased to about 120% of the initial activity and did not correlate with the changes in endogenous cAMP level. The 2-fold increase of protein kinase activity during 1 min of incubation with 5-HT was apparently not due to activation of cAMP-dependent protein kinase. This increased activity of the particulate fraction could not be obtained by the addition of cAMP to fractions from heads incubated in the absence of 5-HT. The increased enzyme activity could not have been due to transfer of activity from the supernatant fraction, since during this minute no change in the amount of activity in the supernatant fraction was observed (see Fig. 3A). Thus the effect of 5-HT on protein kinase activity in the particulate fraction may involve other means in addition to activation by cAMP.

The relationship between the degree of activation of protein kinase by cAMP and the level of endogenous cAMP is shown in Fig. 3C. The degree of activation of both fractions decreased during the first 5 min of incubation and then increased during

the next 15 min. These changes in the degree of activation by cAMP corresponded inversely with the changes in the level of endogenous cAMP. The results provide further evidence for the role of cAMP as a mediator of the activation of protein kinase by 5-HT.

LSD antagonism of 5-HT activation of protein kinase. The effect of LSD on 5-HT activation of protein kinase in fluke heads is shown in Table 4. The values in this table are presented as the ratio of the enzyme activity in heads incubated with 5-HT and/or LSD to the enzyme activity in heads incubated in the saline solution. In Table 4, 5-HT is shown to cause an increase in protein kinase activity as described above. When fluke heads were incubated with 10 μM LSD alone, no significant effect on protein kinase activity was observed. However, when LSD was added to incubation solutions containing 1 mM 5-HT, a reduction of the 5-HT activation of protein kinase was seen. This antagonism of 5-HT by LSD was significant except in the supernatant fraction assayed without cAMP. The order of addition of 5-HT and of LSD made no difference in the protein kinase activity of either fraction. LSD, which mimics some of the effects of 5-HT on the fluke (11, 13, 15), has been shown to antagonize the 5-HT-stimulated increase in endogenous cAMP (15). It appears from these data that LSD antagonized the activation of protein kinase by 5-HT through its effects on adenylate cyclase and endogenous cAMP accumulation. When added directly to the protein kinase assay solution, neither LSD nor 5-HT had any effect on protein kinase activity.

Phosphorylation in particulate and supernatant fractions of fluke head homogenates. In the protein kinase assays done in this study, we used blanks which contained fluke homogenate fractions but not protamine. It was noted that a significant amount of phosphate (about 25% of that bound to protamine) was incorporated into the trichloroacetic acid precipitate from these blanks. Seventy per cent of the radioactive phosphate in this precipitate could not be extracted with hot 5% trichloroacetic acid or ethanol–ether, indicating that the phosphate was bound to protein rather than polynucleotides or phospholipids. Thus the incorporation of phosphate in the absence of protamine appears to be due to protein kinase activity using fluke protein as the substrate. Both particulate and supernatant fractions assayed in the presence of 5 μM cAMP showed increased phosphate incorporation into fluke protein. The mean activation by cAMP (± standard error) was 1.4 ± 0.1-fold in the particulate

TABLE 4

Effect of LSD on 5-HT activation of protein kinase

Fluke heads were incubated with 1 mM 5-HT and 10 μM LSD, either separately or in combination. The heads were homogenized, centrifuged, and assayed for protein kinase activity as described in Table 1. The protein kinase activity of these heads was always compared with that from fluke heads incubated with no additions in the same experiment. Values are expressed as the ratio of protein kinase activity after incubation with effectors to the activity obtained in the absence of effector.

Addition to incubation solution	No. experiments	Protein kinase activity			
		Particulate fraction		Supernatant fraction	
		Without cAMP	With cAMP	Without cAMP	With cAMP
		× *control* ± *SEM*		× *control* ± *SEM*	
1 mM 5-HT	11	4.25 ± 0.81	1.82 ± 0.22	1.41 ± 0.23	0.64 ± 0.10
10 μM LSD	4	0.96 ± 0.20[a]	1.20 ± 0.13[a]	1.05 ± 0.09[a]	1.32 ± 0.16[a]
1 mM 5-HT, 10 μM LSD	6	1.55 ± 0.24[b]	1.64 ± 0.13[b]	1.48 ± 0.23	1.38 ± 0.04[b]

[a] Not significantly different from untreated flukes by the *t*-test.

[b] Significantly different from values obtained with 5-HT ($p < 0.05$ by the *t*-test).

fraction and 2.5 ± 0.7-fold in the supernatant fraction (N = 10). Incubation of the heads with 5-HT resulted in a decreased degree of activation by cAMP (1.2 ± 0.2-fold in the particulate fraction and 1.1 ± 0.1-fold in the supernatant fraction). Incubation with 5-HT also caused a 50% increase in the specific activity of the particulate fraction assayed in the presence of cAMP, but caused no significant change in the activity of the supernatant. As shown in Table 2, when protamine was used as the substrate, a similar reduction in the degree of activation by cAMP and an increase in the particulate activity were found after incubation of the fluke heads with 5-HT. These results suggest that the activity of protein kinase using protamine as a substrate is a valid model for activity toward its natural substrate(s) in the fluke.

DISCUSSION

cAMP-dependent protein kinases have been demonstrated in a variety of invertebrate species (21). For example, the trematode *Schistosoma mansoni* contains this enzyme as well as adenylate cyclase and cAMP phosphodiesterase (22). Hormonal regulation of the protein kinase in invertebrate tissues has not been reported. In previous studies Mansour *et al.* (14) reported that 5-HT activated glycogen phosphorylase and adenylate cyclase in *Fasciola hepatica*. In the accompanying paper (15) it was shown that incubation of the liver fluke with 5-HT increased the endogenous level of cAMP. In this paper we have demonstrated a cAMP-dependent protein kinase which is activated upon incubation of the tissue with 5-HT. The activation of protein kinase by 5-HT appears to be mediated by activation of adenylate cyclase. This is supported by the finding that the activation of protein kinase was accompanied by a marked increase in the endogenous cAMP level. Second, the decreased degree of activation of protein kinase by cAMP after incubation with 5-HT was strongly correlated with the endogenous cAMP level. Third, LSD, which was shown to antagonize 5-HT activation of adenylate cyclase, also antagonized activation of protein kinase. These results provide further evidence for the role of cAMP as a mediator of the effects of 5-HT in the liver fluke. Activation of the protein kinase by cAMP was demonstrated with fluke protein as well as with protamine as the substrate. Phosphorylation of these endogenous proteins could alter their functional state, thus producing the physiological effects of 5-HT in the fluke.

The presence of cAMP-dependent protein kinase in both the supernatant and particulate fractions of fluke homogenates is similar to that reported in many other tissues. The range of cAMP concentrations required for activation of the protein kinase in both homogenate fractions is comparable to that reported for this enzyme from other organisms (17, 22) and could be achieved intracellularly in the fluke following incubation with 5-HT (15). The apparent loss of activity from the supernatant fraction after 5 min of incubation with 5-HT is similar to that reported in response to various other effectors in several mammalian tissues (2–5, 23). For example, a time-dependent decrease in the protein kinase activity of the supernatant fraction from rat liver was noted in response to glucagon (5). Keely *et al.* (24) have presented evidence for translocation of protein kinase in rat heart. Their results indicate that the translocation of the enzyme is due to preferential binding of the kinase catalytic subunit to the particulate fraction under conditions of low ionic strength during homogenization. A similar interpretation can be made from the results with the liver fluke, since redistribution of enzyme activity was obtained by homogenization in low ionic strength buffer containing 5 μM cAMP.

In several tissues the presence of cAMP-dependent protein kinase in the particulate cell fraction has been correlated with regulation of ion transport and membrane permeability (6–9, 25–27). For example, in bovine cardiac muscle (9, 25) and in rat liver (26), protein kinases phosphorylate specific proteins which are implicated in calcium uptake. Control of protein kinases, resulting in dephosphorylation of membrane proteins involved in sodium and potassium transport, has also been reported (7, 27). In several invertebrates, 5-HT and

cAMP have been shown to alter membrane permeability to specific ions (28, 29). We have shown that fluke protein is phosphorylated by cAMP-dependent protein kinase in the particulate fraction. Thus, in the liver fluke, one of the results of 5-HT activation of protein kinase could be regulation of ion transport. Characterization of the natural substrate(s) of protein kinase should further elucidate the mechanism of 5-HT stimulation of motility (10) and metabolism (16) in the liver fluke.

LSD mimics the effects of 5-HT on fluke motility and carbohydrate metabolism (11, 13) but has been shown to antagonize the activation of adenylate cyclase by 5-HT (15). Furthermore, LSD blocks activation of protein kinase by 5-HT, apparently through antagonism of adenylate cyclase. Thus the effects of LSD on the motility and metabolism of the liver fluke do not appear to be mediated by cAMP-dependent protein kinase.

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