Biochemical Pharmacology, Vol. 33, No. 17, pp. 2799–2805, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

DESENSITIZATION OF SEROTONIN-STIMULATED ADENYLATE CYCLASE IN THE LIVER FLUKE *FASCIOLA HEPATICA**

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(*Received* 1 *October* 1983; *accepted* 15 *February* 1984)

Abstract—Incubation of the intact liver fluke *Fasciola hepatica* with serotonin (5-HT) resulted in a time-dependent decrease in both 5-HT-stimulated adenylate cyclase activity and specific [^{3}H]LSD binding in the subsequently prepared cell-free fluke particles. In control fluke particles, the activation of adenylate cyclase by 5-HT was biphasic, indicating a high and low affinity form of the 5-HT receptor with half-maximal activation constants (K_A) of 0.35 and 2.8 μM respectively. In contrast, 5-HT activation of desensitized particles occurred through a single set of low affinity sites having a K_A value of 6.3 μM. The maximal level of 5-HT activation of adenylate cyclase was also reduced in the desensitized particles. Lysergic acid diethylamide (LSD)-stimulated adenylate cyclase activity was also less in the desensitized particles. However, unlike with 5-HT, activation by LSD occurred through a single set of sites for both control and desensitized particles. [^{3}H]LSD binding studies showed that the affinity of LSD for the 5-HT receptors in the control and desensitized particles was similar. Thus, the decrease in [^{3}H]LSD binding and serotonin-stimulated adenylate cyclase activity in the desensitized particles appeared to be due to a preferential loss or inactivation of the high affinity form of the 5-HT receptor. A similar time-dependent loss in 5-HT-stimulated adenylate cyclase and in [^{3}H]LSD binding occurred in cell-free fluke particles upon the addition of 5-HT or LSD. These effects were not due to protein denaturation or metabolism of the ligand during the incubation procedure. This cell-free desensitization was reversible and temperature dependent, and was not affected by ATP or other nucleotides.

Most hormone-sensitive cells have regulatory mechanisms to modulate their responsiveness to these activating agents. Prolonged exposure of a cell to a specific hormone leads to a reduction in the cellular response to that hormone. This process, known as desensitization, has been observed in many cells which respond to hormonal stimulation through activation of adenylate cyclase and the production of cAMP [1]. Highly specific radioligands have made it possible to directly examine changes in the receptor properties of desensitized cells and to compare them with the changes in adenylate cyclase activity. Several such studies on adenylate cyclase coupled β-adrenergic receptors indicated that the decreased responsiveness of hormone-stimulated adenylate cyclase in desensitized cells resulted from the loss of functional β-receptors in these cells [2–5]. Other reports indicated that, in addition to receptor loss, alterations in non-receptor components of the adenylate cyclase system were also involved in the desensitization response [6, 7]. A mechanism of desensitization of adenylate cyclase was also described that required protein synthesis and did not result in a loss of receptors [8, 9]. Such diverse findings suggest that cells may employ several different mechanisms in order to reduce their hormonal responsiveness.

Attempts to study desensitization in cell-free systems have been made recently [10–14]. These reports focused on systems where desensitization of hormone-stimulated adenylate cyclase activity was very rapid and not directly associated with a loss of hormone receptors. Due to differences in experimental conditions, it has been difficult to determine if the mechanisms of cell-free desensitization are related to the desensitization seen in intact cells. Nevertheless, it appears that cells do have mechanisms of hormonal regulation that can be studied in both intact cells and in broken cell preparations.

We previously reported that the incubation of whole flukes with 5-HT‡ resulted in a rapid accumulation of cAMP [15]. cAMP levels reached a maximum within 5–10 min, after which they decreased back to control values despite continued incubation in 5-HT. To study this desensitization process in more detail, we examined both the adenylate cyclase activity and the [^{3}H]LSD binding properties in fluke particles prepared from desensitized flukes. Furthermore, the properties of desensitization in cell-free fluke particles are described and compared with the desensitization seen in intact flukes.

* This investigation was supported by U.S. Public Health Service Grants MH 23464 and AI 16501. These studies constitute a part of the work submitted to the Graduate School, Stanford University, by S. J. M. in partial fulfillment of the requirement for the degree of Doctor of Philosophy. S. J. M. was a recipient of a predoctoral trainee fellowship, U.S. Public Health Service, Training Grant GM 07149.

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‡ Abbreviations: 5-HT: serotonin, 5-hydroxytryptamine; LSD: *d*-lysergic acid diethylamide; K_A: activation constant; Gpp(NH)p: 5′-guanylyl imidophosphate; and App(NH)p: 5′-adenylyl imidophosphate.

METHODS AND MATERIALS

Preparation of desensitized fluke particles. Fresh liver flukes were obtained from bovine livers at a local slaughterhouse and maintained overnight as previouly described [15]. Unless otherwise noted, intact flukes were desensitized to 5-HT by their incubation at 37° in medium containing 1 mM 5-HT. The time of incubation varied and is indicated for each experiment. Following incubation, the flukes were placed in fresh medium not containing 5-HT and stirred, and then the medium was poured off. This washing procedure was repeated twice with fresh medium. The flukes were then blotted dry, their wet weight was determined, and 6 vol. (w:v) of homogenization buffer (0.25 M sucrose, 5 mM dithiothreitol, and 1 mM EDTA) was added. Fluke particles were then prepared by the procedure described in the accompanying paper [16]. The final particles were used directly or kept in liquid nitrogen or in a −70° freezer until just prior to use.

[^{3}H]LSD binding assay. The binding of LSD to the fluke particles was determined by incubating fluke particles (1–3 mg protein/ml, final concentration) with 10 nM [^{3}H]LSD and other effectors as indicated. Unless otherwise stated, the incubation conditions were for 5 min at 37°. Following incubation, [^{3}H]LSD binding was determined by the binding filtration assay described previously [16]. Non-specific binding was determined by the amount of [^{3}H]LSD bound in the presence of 100 μM non-radioactive LSD. Specific binding was determined by subtracting the amount of non-specific binding from the total amount of [^{3}H]LSD bound. All values given are the average of triplicate samples.

Adenylate cyclase assay. Adenylate cyclase activity of fluke particles was determined by a modification of the procedure of Salomon *et al.* [17], as described previously [16], with a reaction mixture containing (final concentrations) 0.1 M sucrose, 20 μM EGTA, 50 mM glycylglycine, pH 7.5, 5 mM phosphocreatine, 2 mM $MgCl_2$, 0.1 mM sodium-ATP with 0.75 to 1.5 μCi [α-^{32}P]ATP, 5 units creatine phosphokinase, 0.5 mM 3-isobutyl-1-methylxanthine, and other compounds as indicated in a final volume of 0.25 ml. The samples were incubated for 5 min at 37° unless otherwise noted. The values given are the average of duplicate samples.

Protein concentrations were determined by the method of Bradford [18] using bovine serum albumin as a standard.

Materials. The materials used in this study were of at least reagent grade and were obtained from sources described previously [16].

RESULTS

Effects of incubation of intact liver flukes with 5-HT on adenylate cyclase activity. To investigate the 5-HT-induced desensitization response of intact flukes, we first measured the activation of adenylate cyclase by 5-HT in particles prepared from flukes that had been incubated with 1 mM 5-HT for various lengths of time prior to their homogenization (desensitized particles). As shown in Table 1, 5-HT-stimulated adenylate cyclase activity in the desensitized particles was significantly less than the 5-HT-stimulated activity in the control particles. The decreased adenylate cyclase activity in the desensitized particles was time-dependent and specific for 5-HT-mediated stimulation as reductions in basal or fluoride-stimulated activity did not occur. Figure 1 compares the kinetics of 5-HT activation of adenylate cyclase in the desensitized and control fluke particles. Maximum 5-HT-stimulated adenylate cyclase activity in the desensitized particles reached only 65–70% of the maximal value of 5-HT stimulation in the control particles. Lineweaver–Burk analysis of the activation data is shown in Fig. 2. For the desensitized particles, activation by 5-HT appeared to occur through a single set of sites with a graphically determined half-maximal activation

Table 1. Desensitization of adenylate cyclase in whole flukes after incubation with 5-HT*

	Incubation time (min)	Adenylate cyclase activity (pmoles/mg·min) Basal	100 μM 5-HT + 100 μM GTP	10 mM NaF	[^{3}H]LSD binding (fmoles/mg protein)
I	0 ("control particles")	6.2	440	2400	38
	10	7.8	350	2200	28
	30	9.4	270	2100	18
II	0	2.4	400	1600	26
	30	4.6	240	1500	13
	60	4.3	210	1700	14
III	0	5.4	340	1300	ND†
	10	15	310	1600	
	30	9.1	220	1200	

* Whole flukes were incubated with 1 mM 5-HT for up to 60 min before being prepared into fluke particles as described in Methods and Materials. Adenylate cyclase activity of the particles was determined as described in Methods and Materials with the indicated compounds added to the reaction mixture at the final concentrations given. The results presented are the means of duplicate samples in three separate experiments. Specific [^{3}H]LSD binding was determined as described in Methods and Materials after incubation for 5 min at 37°. The results given are the means of triplicate samples.

† [^{3}H]LSD binding was not determined.

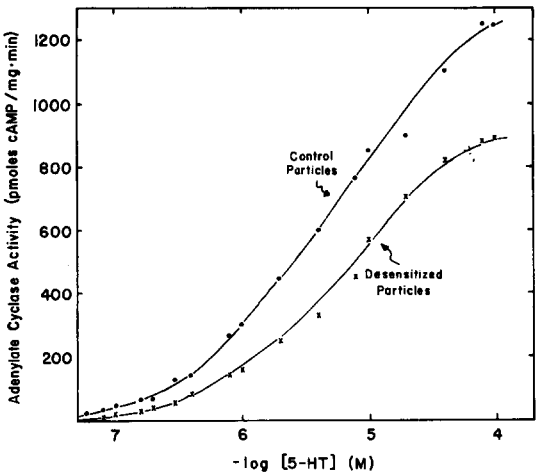

Fig. 1. 5-HT activation of adenylate cyclase in desensitized and control fluke particles. Fluke particles were prepared as described in Methods and Materials from intact flukes that had been incubated with no additions (●) or with 1 mM 5-HT (×) for 30 min at 37°. Adenylate cyclase activity was then determined as described in Methods and Materials with the indicated concentrations of 5-HT and 100 μM GTP present in the reaction mixture. The basal activity (enzyme activity in the absence of 5-HT) has been subtracted from all the values shown. The data are the means of duplicate samples in a single representative experiment.

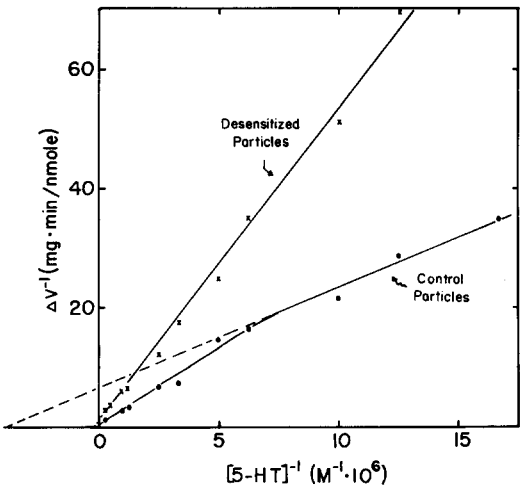

Fig. 2. Lineweaver–Burk plot of 5-HT activation of desensitized and control fluke particles. The data presented in Fig. 1 are represented in a Lineweaver–Burk plot for the desensitized (×) and for the control (●) fluke particles. The line shown for the desensitized particles was determined by linear regression of all the data and gave a half-maximal activation constant (K_A) value of 6.2 μM ($r = 0.998$). Data points for 5-HT concentrations greater than 8 μM have been omitted from the figure for clarity.

constant (K_A) of 6.2 μM. This was in contrast to the control particles where, as previously reported [19], the activation of adenylate cyclase by 5-HT was biphasic, with the two apparent K_A values determined to be 0.35 and 2.8 μM. Thus, in the desensitized particles there was a decrease in both the maximal level of 5-HT-stimulated adenylate cyclase and in the affinity of 5-HT to activate the enzyme.

LSD activation of adenylate cyclase in desensitized particles was also reduced compared to control particles (Fig. 3). As shown in Fig. 4, Lineweaver–Burk analysis of LSD activation indicated a single set of sites for both control and desensitized particles. Both the maximal level of LSD activation of adenylate cyclase and the affinity of LSD for activating the enzyme were reduced in the desensitized particles. In both respects, however, the reduction was less than that seen with 5-HT.

[^{3}H]LSD binding to 5-HT desensitized fluke particles. In the preceding paper [16] a good correlation

Table 2. Effect of incubation time on LSD binding and adenylate cyclase activation*

Incubation time (min)	[^{3}H]LSD binding (% of maximum)	Adenylate cyclase activity (% of maximum)	
		Incubated particles	Supernatant + fresh particles
0	100	100	100
5	84	85	99
15	43	55	84
30	20	35	80
45	17	20	78

* Fluke particles were incubated with 100 nM [^{3}H]LSD and, at the times indicated, an aliquot was removed and specific [^{3}H]LSD binding was determined. Another aliquot was assayed for adenylate cyclase activity, as described in Methods and Materials, in a reaction mixture containing 100 μM GTP. The remainder of the sample was spun at 15,000 *g*, and the supernatant fraction was tested for its ability to activate fresh particles by adding it to an adenylate cyclase reaction mixture containing 100 μM GTP and fresh fluke particles. Adenylate cyclase activity in the supernatant fractions alone was less than 2% of activity measured with the addition of fresh particles. The data presented are the mean values of two separate experiments.

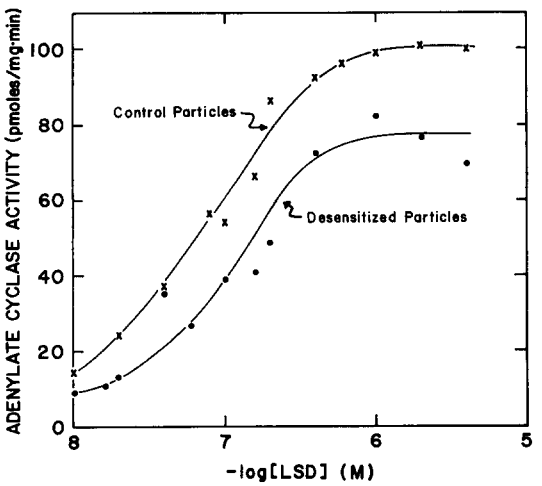

Fig. 3. LSD activation of adenylate cyclase in desensitized and control fluke particles. Desensitized (●) and control (×) fluke particles were prepared as described in Fig. 1. Adenylate cyclase activity was then determined as described in Methods and Materials with the indicated concentrations of LSD and 100 μM GTP present in the reaction mixture. The data represent the means of duplicate samples in a single representative experiment.

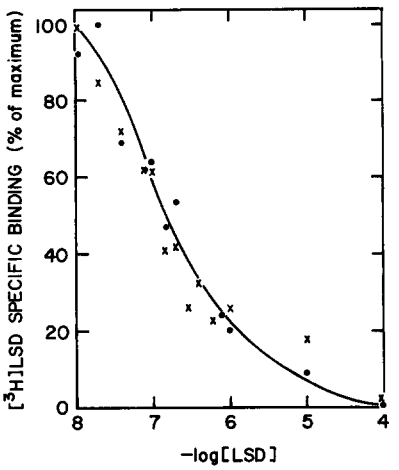

Fig. 5. Displacement of [^{3}H]LSD binding in desensitized and control fluke particles. Desensitized fluke particles were prepared from intact flukes which had been incubated with 1 mM 5-HT for 30 min as described in Methods and Materials. The desensitized fluke particles (●) and the control fluke particles (×) were incubated with 10 nM [^{3}H] LSD and the indicated concentrations of non-radioactive LSD for 5 min at 37°. [^{3}H]LSD specific binding was then determined as described in Methods and Materials.

was found between 5-HT receptor-mediated activation of adenylate cyclase and the specific binding of [^{3}H]LSD to fluke particles. It was thus of interest to determine the characteristics of [^{3}H]LSD binding to the 5-HT desensitized fluke particles. Table 1 shows that the desensitized particles bound less [^{3}H] LSD than control particles under identical conditions. This decrease in [^{3}H]LSD binding did not appear to be due to residual 5-HT being carried over from the intact organisms to the particles. Incubation of intact flukes with [^{3}H]5-HT indicated that, in the subsequently prepared particles used to measure [^{3}H] LSD binding, the concentration of carried over 5-HT was less than 200 nM. The results from the accompanying paper [16] showed that this concentration of 5-HT would not affect [^{3}H]LSD binding

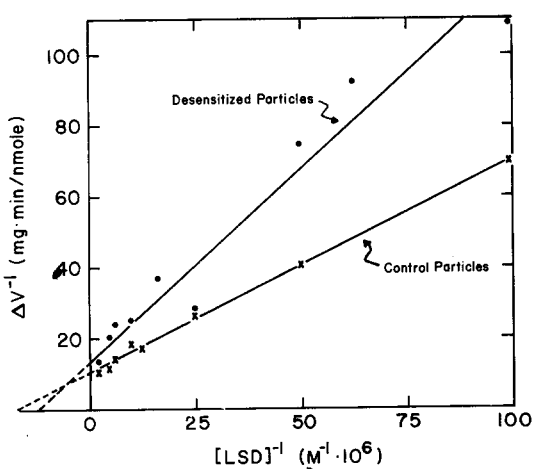

Fig. 4. Lineweaver–Burk plot of LSD activation in desensitized and control fluke particles. The data in Fig. 3 are shown in a Lineweaver–Burk plot for LSD activation in desensitized (●) and in control (×) fluke particles. The lines shown were determined by linear regression of all the data shown in Fig. 3. This analysis gave for the desensitized particles: K_A = 84 nM, V_{max} = 77 pmoles/mg·min (r = 0.976); and for the control particles: K_A = 60 nM, V_{max} = 100 pmoles/mg·min (r = 0.998). The data points for LSD concentrations greater than 400 nM have not been included in the figure for clarity.

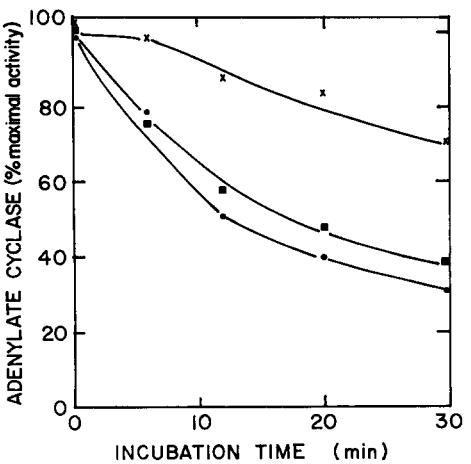

Fig. 6. Time course of adenylate cyclase activation in liver fluke particles at 37°. Fluke particles from the same preparation used in Fig. 2 were incubated at 37° with 10 μM 5-HT + 100 μM GTP (■), 1 μM LSD + 100 μM GTP (●), or 10 mM sodium fluoride (×). At the times indicated, aliquots were added to a reaction mixture and adenylate cyclase activity was determined as described in Methods and Materials. Assay time was for 3 min at 37°. The results are the means of duplicate assays. The maximum cyclase activities were 1,530, 340, and 10,400 pmoles of cAMP per ml per min for 5-HT, LSD, and sodium fluoride respectively.

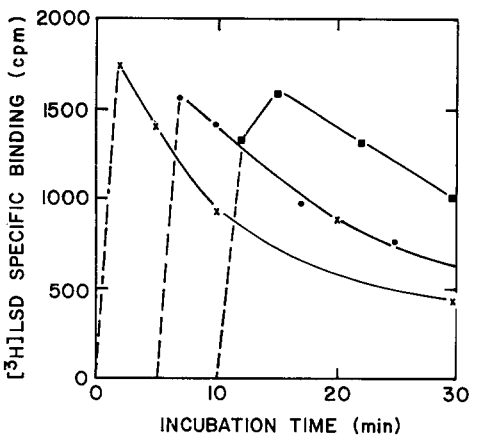

Fig. 7. Effect of preincubation on [^{3}H]LSD binding to liver fluke particles. Fluke particles were incubated with 10 nM [^{3}H]LSD at 30° after preincubation for 0 (×), 5 (●), or 10 (■) min at 30° without ligand. At the times indicated, specific [^{3}H]LSD binding was determined as described in Methods and Materials. The data shown are the means of triplicate assays in a single representative experiment.

to the extent seen. We cannot, however, exclude the possibility that some of the decrease in [^{3}H]LSD binding in the desensitized particles was the result of 5-HT remaining tightly bound to the [^{3}H]LSD binding sites.

An attempt was made to determine if the reduction in [^{3}H]LSD binding was the result of lower affinity of LSD for the receptors or due to an actual decrease in receptor number. The ability of unlabeled LSD to displace [^{3}H]LSD binding was compared in control and desensitized fluke particles. As shown in Fig. 5, the affinity of LSD for the receptors did not change in the desensitized particles. Thus, the decrease in [^{3}H]LSD binding in the desensitized particles appeared primarily to be due to receptor loss or inactivation. A decrease in the number of receptors in the desensitized particles is consistent with the decreased 5-HT-stimulated adenylate cyclase activity described above.

Cell-free desensitization in fluke particles. We previously reported [16] that the incubation of fluke particles at 37° resulted in a time-dependent decrease in the amount of specific [^{3}H]LSD binding. Figure 6 shows that 5-HT and LSD activation of adenylate cyclase decreased in a similar time-dependent manner. This effect was not due to a time-dependent irreversible denaturation of the enzyme, since adenylate cyclase activity measured at intervals with NaF decreased at a much slower rate than did 5-HT or LSD activation. Since fluoride activation is thought to require only the regulatory and catalytic components of the adenylate cyclase complex, it was used as a measure of the stability of these components during the incubation. The data suggest that the faster decay of 5-HT- and LSD-stimulated adenylate cyclase was due primarily to effects at the receptor site.

The possibility that the reduction in [^{3}H]LSD binding and adenylate cyclase activity was due to nonspecific proteolysis or denaturation of the binding system was tested. Particles were incubated at 37° for different time periods prior to the addition of [^{3}H]LSD and the determination of specific binding. The decrease in [^{3}H]LSD binding during incubation varied with different particle preparations. However, as indicated in Fig. 7, it was consistently found that incubation of fluke particles in the presence of [^{3}H]LSD decreased binding at a more rapid rate than the incubation of the particles alone. Furthermore, the protease inhibitor phenylmethylsulfonylfluoride, at concentrations up to 100 μM and 1 mM EDTA in the incubation buffer, had no effect on the time-dependent decrease of [^{3}H]LSD binding. The possibility that the decrease in binding was the result of the metabolism of the ligand was also tested. Thin-layer chromatography of the [^{3}H]LSD before and after incubation at 37° showed no significant changes. The stability of LSD was also determined by bioassay of LSD on fluke particles. Fluke particles were incubated with LSD at 37° for various time periods and then pelleted by centrifugation. The ability of the LSD remaining in the supernatant fraction to stimulate adenylate cyclase activity was then tested by its addition to untreated fluke particles. The results given in Table 2 show that the LSD in supernatant fractions taken from particles in which the amount of binding and adenylate cyclase activity was markedly reduced retained its ability to stimulate adenylate cyclase in fresh fluke particles. These studies indicated that the destruction of ligand did not account for the decreased [^{3}H]LSD binding and adenylate cyclase activity reported.

As indicated previously [16], the time-dependent decrease in [^{3}H]LSD binding was temperature dependent. Figure 8 shows the time course of binding at 25° and 0°. At 25°, the binding was similar to that reported for 37°, reached a maximum within 5 min, and then steadily decreased. At 0°, however, no

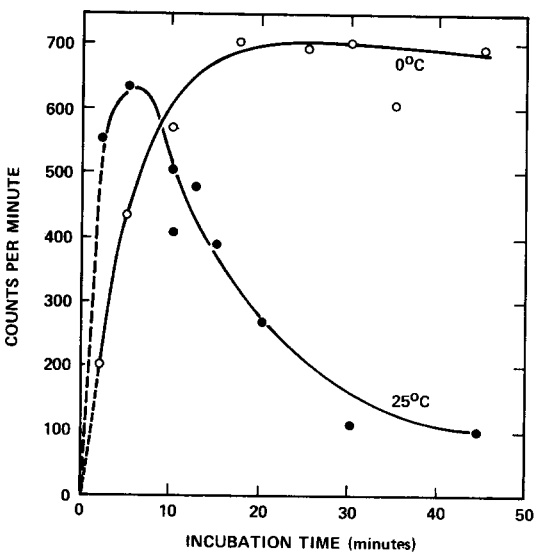

Fig. 8. Time course of [^{3}H]LSD binding to liver fluke particles at 0° and 25°. Fluke particles were incubated with 10 nM [^{3}H]LSD at 0° (○) or 25° (●). At the times indicated, specific [^{3}H]LSD binding was determined as described in Methods and Materials. The data shown are the means of triplicate assays in a single representative experiment.

Table 3. Reversibility of LSD binding in fluke particles*

Treatment of particles	Incubation time (min)	[^{3}H]LSD specific binding (fmoles/mg protein) Expt. 1	Expt. 2	Expt. 3
(A) Incubated + 10 nM [^{3}H]LSD	5	155	219	255
	30	96	36	148
(B) Incubated + 10 nM [^{3}H]LSD, washed, and reincubated with 10 nM [^{3}H]LSD	5	212	270	305
	30	121	99	157
(C) Incubated without 10 nM [^{3}H]LSD, washed, and incubated with 10 nM [^{3}H]LSD	5	228	240	355
	30	138	79	267

* In A, fluke particles were incubated with 10 nM [^{3}H]LSD at 37°, and at the times indicated [^{3}H]LSD specific binding was determined as described in Methods and Materials. In B, particles that were desensitized after incubation with [^{3}H]LSD for 30 min were washed three times with ligand-free homogenizing mixture and their ability to bind [^{3}H]LSD was examined as in A. In C, particles were preincubated without LSD and then were treated as in B before their ability to bind [^{3}H]LSD was examined. The results presented are the means of triplicate samples in three separate experiments.

significant decrease in [^{3}H]LSD binding was seen after 45 min of incubation.

The nature of this response, which will be referred to as cell-free desensitization, was further examined to determine if it was related to the desensitization in intact flukes described earlier. One major difference between the two processes was the concentration of ligand required to elicit the desensitization response. Concentrations of 100 μM to 1 mM of 5-HT were needed for desensitization to occur in the intact flukes. In contrast, cell-free desensitization occurred with as little as 10 mM LSD. Higher concentrations of LSD did not increase the rate or extent of the cell-free desensitization response, indicating that 10 nM LSD induced the maximal response.

Both cell-free desensitization and the desensitization in intact flukes were reversible processes. Table 3 shows that cell-free desensitization was reversed very rapidly following the removal of the desensitization ligand by washing the particles. The results show that addition of fresh ligand to fluke particles that had been desensitized and then washed resulted in binding levels similar to those seen in fluke particles which had not been desensitized previously. Following continued incubation with the ligand, the resensitized particles again became desensitized. In contrast to the rapid reversal of cell-free desensitization, a much longer time period was required to reverse the desensitization process in intact flukes. An extensive time course of the reversal process in intact flukes was not performed; however, it was found that 2–4 hr of incubation in 5-HT medium did not alter the desensitized state of the flukes. Overnight incubation in 5-HT-free medium did return the flukes back to their normal state. The extended time required for the reversal of desensitization in intact flukes suggests that additional cellular steps may be involved that are not present in the rapidly reversed cell-free desensitization.

Inability of various agents to affect cell-free desensitization. Several agents were tested to determine their effect on cell-free desensitization. While addition of GTP was necessary for activation by 5-HT or LSD, addition of concentrations up to 100 μM of GTP or Gpp(NH)p had no effect on cell-free desensitization as measured by [^{3}H]LSD binding. ATP and App(NH)p were also without effect. This is of interest since several reports [11–14] have indicated that one or the other of these nucleotides is necessary for desensitization to occur in other cell-free systems. Other compounds found to have no effect on fluke cell-free desensitization included the reducing agents dithiothreitol and molybdate, the monoamine oxidase inhibitor pargyline, and cholera toxin, which decreased the thermal lability of fluoride activation of adenylate cyclase in fluke particles (unpublished observation). The inability of these compounds to affect cell-free desensitization leaves its mechanism unexplained. It can be stated that the process required the presence of ligand, was temperature dependent, and can be reversed rapidly.

DISCUSSION

The results of this study indicate that in the liver fluke the process of desensitization to 5-HT is characterized by an apparent reduction in the number of 5-HT receptors and a decrease in the affinity of 5-HT for the receptors that do remain. In control particles, 5-HT activation of adenylate cyclase was biphasic, while in desensitized particles 5-HT activation occurred through a single set of low-affinity sites. Activation of adenylate cyclase by LSD was through a single set of sites for both control and desensitized particles. Our previous report [19] that LSD blocked 5-HT activation of adenylate cyclase suggests that LSD cannot distinguish between the high and low affinity 5-HT activation sites. If this interpretation is correct, a decrease in the number of high affinity 5-HT sites should be reflected by a decrease in the maximal level of stimulation of adenylate cyclase by LSD (fewer receptors), but little change in LSD affinity for the remaining receptors. This is consistent with what was observed for LSD activation of adenylate cyclase and for [^{3}H]LSD binding in the desensitized particles.

Desensitization in intact flukes appears similar to the isoproterenol-induced desensitization reported in frog erythrocytes [4, 5]. The desensitized cells showed a decrease in the number of β-adrenergic

receptors and a markedly reduced ability of the remaining receptors to form a high affinity receptor state for isoproterenol. Ying-Fu *et al.* [7] showed that incubation of 1321N1 astrocytoma cells with isoproterenol resulted in a rapid decrease in hormone-stimulated adenylate cyclase activity but no significant change in the number of β-adrenergic receptors for up to 45 min. It was concluded that prior to receptor loss the receptors first passed through an intermediate step in which they were functionally uncoupled from the adenylate cyclase system. Our studies with the fluke indicate that such an uncoupled form of the 5-HT receptor was not present. Particles prepared from flukes which had been incubated with 5-HT for as little as 10 min showed a reduction in both [^{3}H]LSD binding and 5-HT-stimulated adenylate cyclase. Under no conditions was a reduction in 5-HT-stimulated adenylate cyclase activity found without a corresponding decrease in [^{3}H]LSD binding.

A clear relationship was not found between desensitization in intact flukes and cell-free desensitization in fluke particles. Cell-free desensitization occurred in both control particles and in particles prepared from desensitized flukes. The rapid reversibility of the response and the low concentrations of LSD at which cell-free desensitization occurred suggest mechanistic differences between this response and the desensitization seen in intact flukes. Cell-free desensitization was temperature-dependent and it is of interest to note that a similar inhibition of isoproterenol-induced desensitization by low temperature in a cell-free system has been reported [11]. The rapid decrease in both [^{3}H]LSD binding and 5-HT-stimulated adenylate cyclase in fluke cell-free desensitization differed from the finding in other cell-free systems [11, 13, 14] where rapid decreases in hormone-stimulated adenylate cyclase were accompanied by little or no loss of receptors. Furthermore, cell-free desensitization in these systems was dependent upon nucleotide, either ATP or GTP, a condition not required in the fluke particles. Mukherjee and Lefkowitz [20] reported that incubation of frog erythrocyte membranes with isoproterenol led to an apparent loss of receptors. However, subsequent studies [21] showed that the receptors were not actually lost but remained tightly bound to agonist which was not removed by the washing procedure used. A similar explanation for the decreased [^{3}H]LSD binding in fluke particles is not appropriate, since [^{3}H]LSD was present throughout the desensitization process. Instead, the decrease in [^{3}H]LSD binding appears to represent an actual change at the receptor which is responsible for the corresponding decrease in 5-HT-stimulated adenylate cyclase activity. This process appears to represent a novel mechanism of cell-free desensitization not previously described.

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