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THE NATURE OF THE BINDING BETWEEN LSD AND A 5-HT RECEPTOR: A POSSIBLE EXPLANATION FOR HALLUCINOGENIC ACTIVITY

M.J. BERRIDGE & W.T. PRINCE

A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge CB2 3EJ

1 (+)-Lysergic acid diethylamide (LSD) mimicked 5-hydroxytryptamine (5-HT) in its ability to stimulate fluid secretion, to change transepithelial and intracellular potentials as well as to increase the cyclic 3′,5′-adenosine monophosphate (cyclic AMP) concentrations of isolated salivary glands of *Calliphora*.

2 Unlike 5-HT, LSD disengages slowly from the receptor and fluid secretion continues despite repeated washing.

3 Both 5-HT and tryptamine prevented LSD from acting on the glands.

4 LSD bound to the receptor was slowly displaced when glands were treated with agonists (tryptamine) or antagonists (gramine).

5 The property of LSD which permits it to function as an agonist despite remaining tightly bound to the receptor is discussed as a possible basis for its profound effects within the central nervous system.

Introduction

The possibility that 5-hydroxytryptamine (5-HT) functions as a neurotransmitter mediating important control mechanisms in the brain has attracted attention in recent years (Bradley, 1968; Aghajanian, 1972a). Considering the many structural similarities which exist between 5-HT and a range of hallucinogenic molecules (Chothia & Pauling, 1969; Kang & Green, 1970), it is conceivable that the latter act on a common tryptamine receptor within the brain. A common site of action for different hallucinogens is certainly consistent with the observation that molecules of diverse structures, e.g. (+)-lysergic acid diethylamide (LSD), mescaline and psilocin display cross tolerance (Brawley & Duffield, 1972). Although LSD is usually thought to act as a 5-HT antagonist there are numerous cases where it has been found to either mimic or facilitate the action of 5-HT (Greenberg, 1960; Mansour, Sutherland, Rall & Buedling, 1960; Curtis & Davis, 1961; Aghajanian, 1972b). We have used the salivary glands of an insect as a model system to analyze this proposed relationship between the actions of 5-HT and LSD.

Various invertebrate systems have already proved of value in studying the mode of action of 5-HT (Greenberg, 1960; Gerschenfeld, 1971; Berridge, 1972; Walker & Woodruff, 1972). The salivary glands of the blow-fly are particularly useful not only because of their sensitivity to 5-HT but also because their structural simplicity greatly facilitates the interpretation of pharmacological experiments (Berridge & Prince, 1972a). These organs are simple tubes which are not innervated or vascularized, and which lack a thick connective tissue and musculature (Oschman & Berridge, 1970). The glands function well *in vitro* and some of the intermediate steps between activation of the receptor by 5-HT and the onset of fluid secretion have been worked out (Berridge, 1970; Berridge & Prince, 1972a, 1972b; Prince, Berridge & Rasmussen, 1972; Prince & Berridge, 1972, 1973). Structure-activity studies employing a range of indoleamines and phenylethylamines have revealed that the pharmacological properties of the 5-HT receptor on salivary glands are very similar to those found in both invertebrates and mammals (Berridge, 1972). The way in which LSD interacts with this 5-HT receptor has therefore been investigated.

Methods

Salivary glands were obtained from adult female blow-flies, *Calliphora erythrocephala*. The methods of measuring the rate of fluid secretion and the transepithelial and intracellular potentials

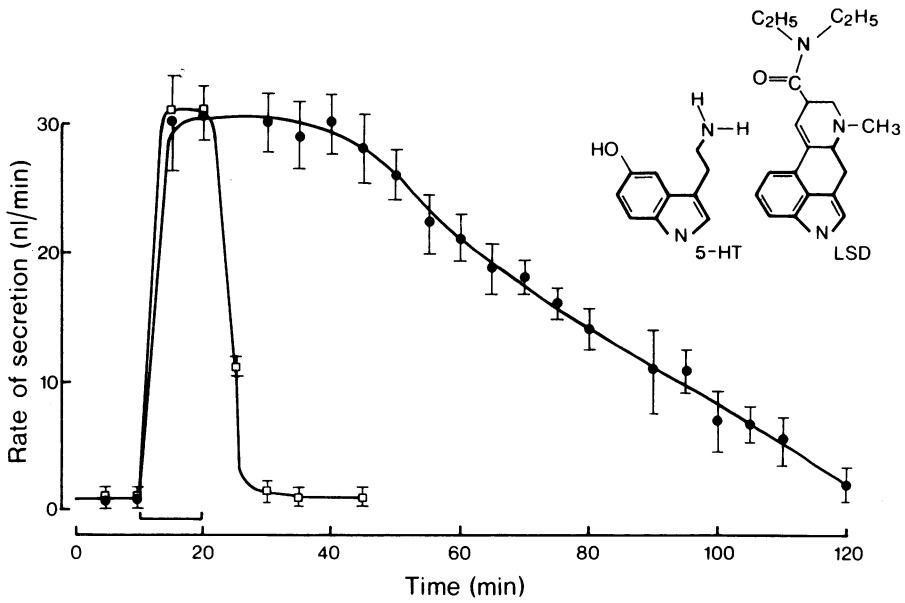

Fig. 1 The effect of 10 nM 5-HT (□) and 10 nM LSD (●) applied for 10 min (bracket) on the rate of fluid secretion by isolated salivary glands. The doses of 5-HT and LSD were chosen so that the onset and maximal responses were similar. The similarity between the structure of 5-HT and LSD is emphasized by the thicker lines in their molecular structures.

have already been described (Berridge & Patel, 1968; Berridge & Prince, 1972b; Prince & Berridge, 1972). The intracellular level of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) was determined using an assay method devised by Gilman (1970) as described previously (Prince *et al.*, 1972). The points on the curves depicting secretory rates represent the means of experiments on at least six salivary glands and the vertical lines indicate twice the standard error of the mean.

The physiological saline used in these experiments had the following composition (mmol/l): Na 155, K 20, Ca 2, Mg 2, Cl 156, phosphate 7, malate 2.7, glutamate 2.7 and glucose 10. In the chloride-free saline, sodium chloride was replaced with sodium isethionate and potassium, calcium and magnesium were introduced as the sulphates. In the calcium-free saline, calcium was omitted from the saline and 5 mmol/l ethylene, glycol-bis-(β-amino ethyl ether)-*N*, *N*′-tetra acetic acid (EGTA) added (Prince & Berridge, 1973). The resulting solution was acidic and was titrated to pH 7.2-7.4 with 1N NaOH before use. EGTA was added to ensure that the saline was completely calcium-free since the salivary glands are sensitive to low concentrations of calcium (1 μM) (Prince & Berridge, 1973). There was no qualitative difference between results obtained with calcium-free salines with or without EGTA. Phenol red (<0.01 mmol/l) was routinely included in all salines to provide a continuous check on the pH.

The compounds used in this study were 5-hydroxytryptamine creatinine sulphate, tryptamine and lysergic acid (Sigma); gramine and harmaline (Koch-Light); (+)-lysergic acid diethylamide tartrate; 2-bromolysergic acid diethylamide (Sandoz).

Results

The effect of (+)-lysergic acid diethylamide (LSD) on the onset of secretion

The response of salivary glands to brief treatment with either 5-hydroxytryptamine (5-HT) or (+)-lysergic acid diethylamide (LSD) is shown in Figure 1. Both induced a rapid onset in secretion which reached a maximal rate within one minute. There was a profound difference in the behaviour of glands when these two agents were removed from the bathing medium. Within 2 min of removing 5-HT the rate of secretion returned to the unstimulated level. On the other hand, after

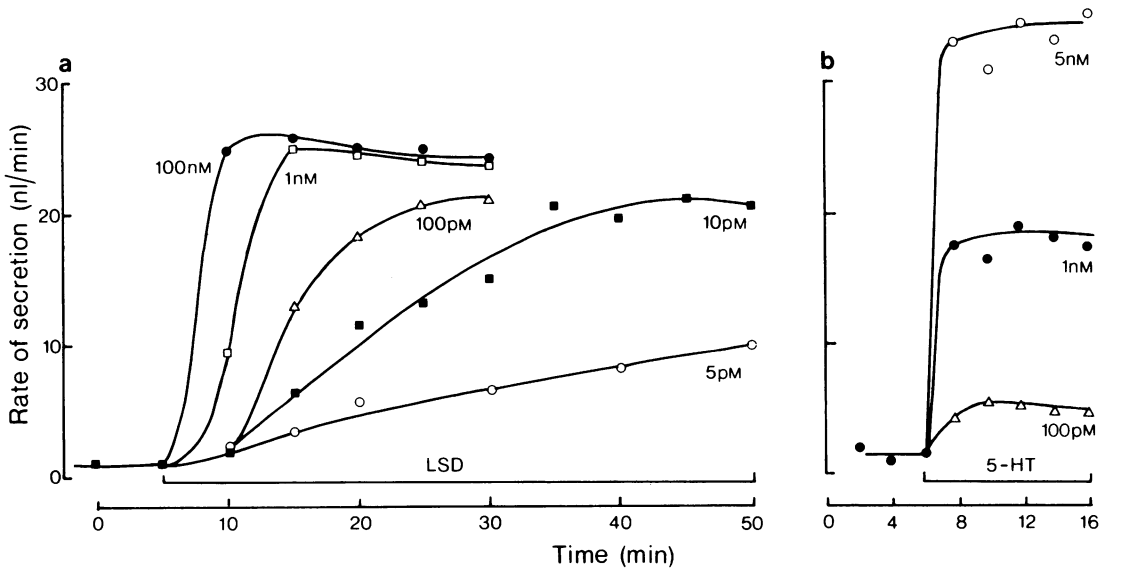

Fig. 2 The effect of different concentrations of LSD (a) or 5-HT (b) on the speed of onset of the secretory response. The period of drug application is shown by the bracket.

removing LSD there was a very slow recovery despite continuously washing the glands to remove every trace of extracellular LSD (Figure 1). LSD thus appears to remain tightly bound to the gland. The binding was not irreversible and eventually the secretory rate decreased to the normal resting rate. After such treatment with LSD, the glands responded normally to 5-HT.

While testing the effect of these different concentrations of LSD it was also noted that the onset of the response was markedly dose-dependent (Figure 2a). At high doses, the onset of the response was similar to that observed with 5-HT. At sub-maximal concentrations, however, the secretory response took much longer to develop (Figure 2a). This is in contrast to the situation with 5-HT where the maximal rate of secretion for all sub-maximal concentrations was achieved in unison (Figure 2b).

Figure 2 also reveals that fluid secretion can be stimulated by doses of LSD far below those of 5-HT. 2-Bromolysergic acid diethylamide can also stimulate fluid secretion and the behaviour of sub-maximal doses was essentially the same as that depicted for LSD in Figure 2a.

Inhibition of (+)-lysergic acid diethylamide (LSD) action by 5-hydroxytryptamine (5-HT) and tryptamine

If LSD acts on the same receptor as 5-HT it should be possible to demonstrate competitive activity between these two agonists. This was done by treating two groups of glands either with LSD alone or together with 5-HT and then following the recovery of secretion when the two groups were washed with control medium (Figure 3a). Glands treated with 1 nM LSD alone showed the typical slow onset of secretion followed by a slow recovery phase. On the other hand, glands treated with both LSD and 5-HT showed a rapid onset of secretion. After removal of the two agonists the rate rapidly fell to a much lower level which then gradually declined towards the unstimulated level (Figure 3a). Similar observations were obtained with tryptamine (Figure 3b). This agonist, which is less active than 5-HT in stimulating fluid secretion (Berridge, 1972), was also less effective than 5-HT in preventing the action of LSD. When compared with 5-HT, equimolar concentrations (1 μM) of tryptamine provided much less protection against LSD (Fig. 3a and b) but increasing the concentration of tryptamine to 100 μM, however, provided more complete protection thus confirming the view that LSD and tryptamine are competing for the same site.

Displacement of (+)-lysergic acid diethylamide (LSD) from the receptor with agonists and antagonists

The following experiments tested the ability of agonists and antagonists to compete with LSD once the latter had attached itself to the receptor

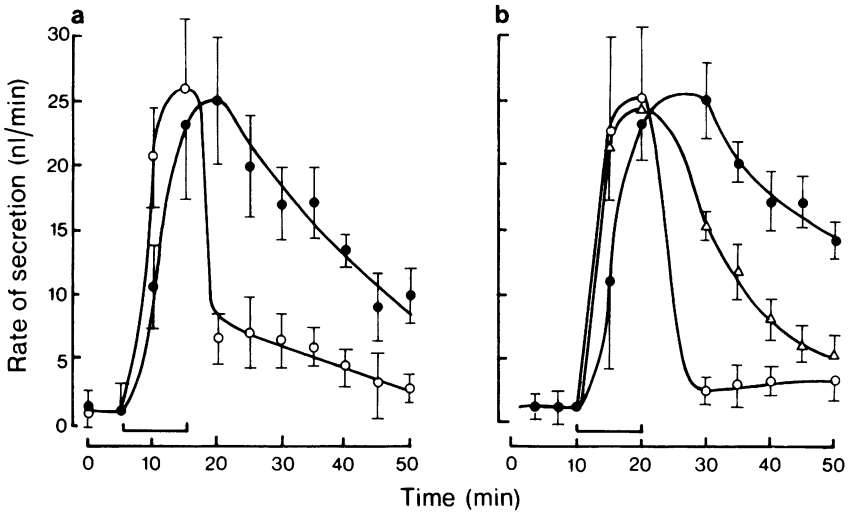

Fig. 3 The effect of 5-HT and tryptamine on the response of salivary glands to LSD. (a) During the period indicated by the bracket a control group of salivary glands was treated with 1 nM LSD (•) whereas a second group was treated with 1 nM LSD together with 1 μM 5-HT (○). After these treatments both groups were returned to control medium. (b) The experimental protocol was similar to that in (a). Glands were treated during the period indicated by the bracket as follows: 1 nM LSD (•); 1 nM LSD + 1 μM tryptamine (△); 1 nM LSD + 100 μM tryptamine (○).

site responsible for stimulating secretion. The recovery of glands which had been treated for 10 min with 1 μM LSD was insensitive to subsequent treatment with 100 μM gramine or harmaline for 15 min (Figure 4). Similar concentrations of gramine or harmaline caused immediate and reversible inhibition of fluid secretion in salivary glands stimulated continuously with 5-HT. The glands which had been pretreated with LSD were totally insensitive to harmaline even after being washed in control medium for 30 min (Figure 4).

Although glands which have been pretreated with LSD appear to be insensitive to the immediate action of 5-HT antagonists, prolonged treatment with gramine caused a gradual inhibition of secretion (Figure 5a). If such glands which had been treated with gramine were returned to control medium the rate of secretion remained at the unstimulated level. Normal increases in the rate of fluid secretion were induced if such glands were once again treated with either LSD or 5-HT.

Agonists are also capable of displacing LSD. Twelve glands were stimulated with LSD for 5 min and six glands were then washed in control medium and showed the typical very slow recovery (Figure 5b). The remaining six glands were treated for 30 min with 100 μM tryptamine before being washed with control medium. The rate of secretion of these glands immediately dropped to a low level when placed in control medium (Figure 5b).

The effect of lysergic acid

Lysergic acid can also stimulate fluid secretion. Unlike LSD the recovery from lysergic acid treatment resembled that of 5-HT because the response recovered rapidly when the glands were returned to control medium. However, the action of lysergic acid was somewhat anomalous, because the dose-response curve was very flat which suggests that lysergic acid is a partial agonist. The gland began to respond at 10 nM lysergic acid but the maximal response was not achieved until the concentration was raised to 100 μM. On the other hand, 5-HT attained a maximal response over approximately one log unit (Figure 2b).

The effect of (+)-lysergic acid diethylamide (LSD) on the transepithelial potential of isolated salivary glands

The transepithelial potential was investigated in an effort to obtain more conclusive evidence that LSD induces secretion by the same mechanism as 5-HT.

The responses of a salivary gland to a short

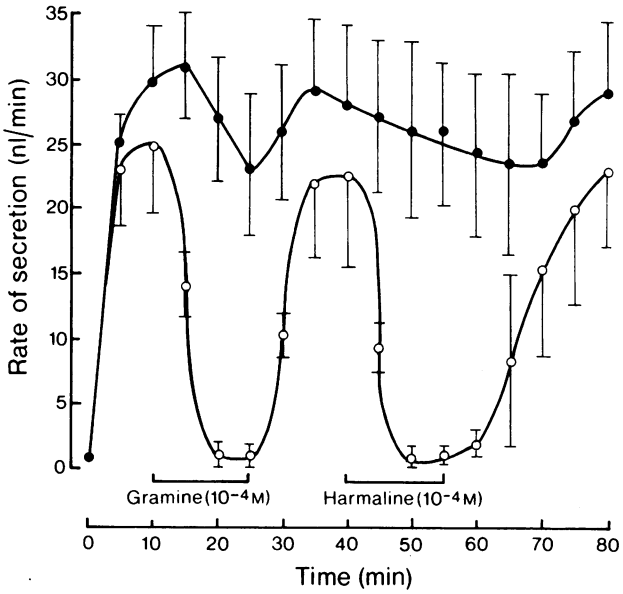

Fig. 4 The effect of gramine and harmaline (the brackets represent the periods of treatment) on the rate of fluid secretion of salivary glands stimulated for a short initial period (0-10 min) with 1 μM LSD (•) or continuously with 10 nM 5-HT (○).

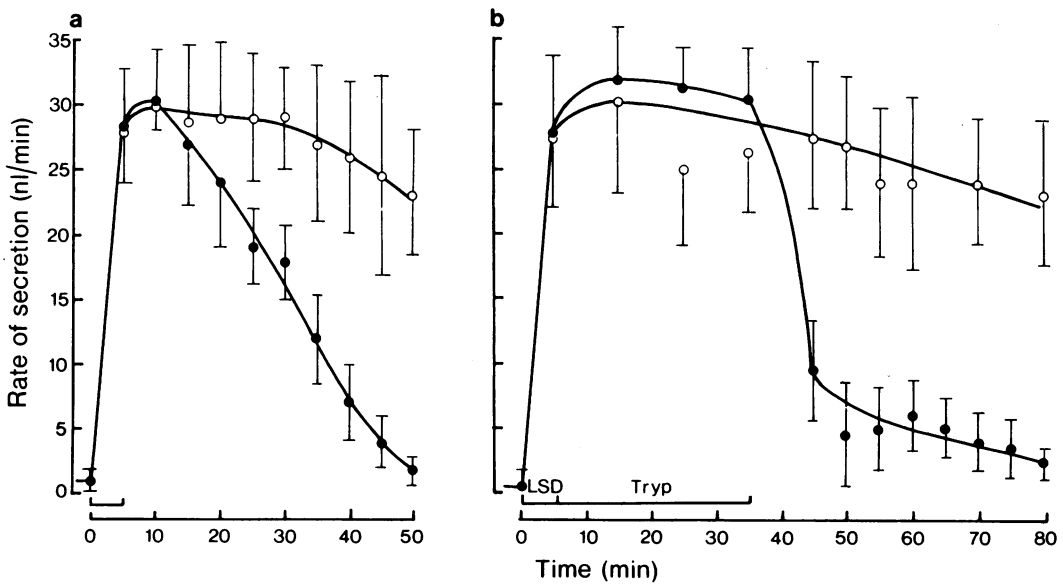

Fig. 5 The effect of gramine (a) or tryptamine (b) on the recovery of isolated salivary glands which have been treated with LSD. (a) After being stimulated with 1 μM LSD (bracket) one group of glands was washed in control medium (○) whereas the second group was washed with 100 μM gramine (•). (b) After being stimulated with 1 μM LSD one group of glands was washed in control medium (○) whereas the second group was washed with 100 μM tryptamine (•) for 30 min before being washed with the control medium.

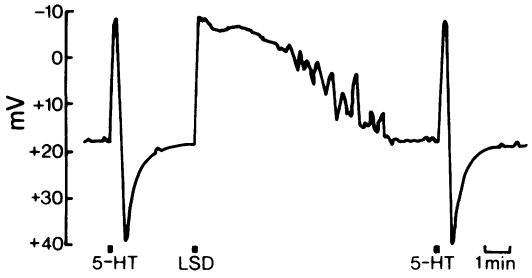

Fig. 6 Changes in the transepithelial potential (lumen relative to bathing medium) produced by 10 s treatments (solid bars) with either 10 nM 5-HT or 10 nM LSD.

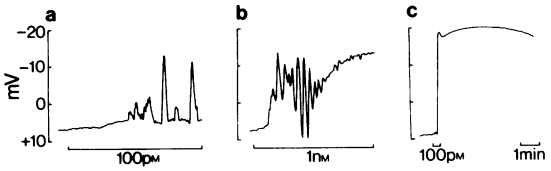

Fig. 7 The effect of different concentrations of LSD on the transepithelial potential of isolated salivary glands.

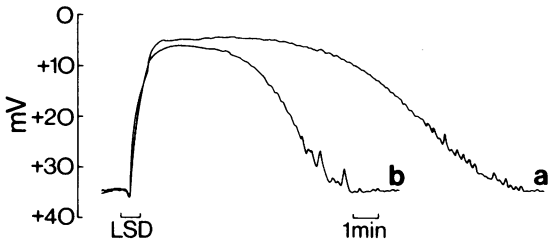

Fig. 8 The effect of gramine on the recovery of the transepithelial potential response to 10 nM LSD. After treatment with LSD (bracket) the gland was washed with either control medium (a) or with a medium containing 100 μM gramine (b). For comparison the two electrical records have been superimposed.

(10 s) treatment of 5-HT showed a characteristic negative phase (lumen relative to blood) which was followed by a positive phase (Figure 6). Such responses have been described previously and are very reproducible (Berridge & Prince, 1972a). However, when glands were treated with 10 nM LSD for the same period of time the potential went negative and remained negative for several minutes before gradually returning to the resting level. The shape of this response resembles those produced by long treatments with 5-HT when no positive phase is seen (Berridge & Prince, 1972b). The recovery of the LSD response shown in Fig. 6 takes over 8 min before it approaches the resting level. If glands were treated for longer periods then the recovery was also longer, e.g. a gland treated for 1 min took 16 min to recover. As with 5-HT, the response of the basal membrane (i.e. the change in potential of a microelectrode recording the cellular potential with reference to the perfusion bath) to treatment with LSD was a slow hyperpolarization of up to 6 mV which returned to the resting level as the transepithelial potential recovered.

The potential responses seen on the addition of LSD in the absence of calcium or chloride were similar to those produced by 5-HT in these same media (Berridge & Prince, 1972a; Prince & Berridge, 1973). When 10 nM LSD was added to a gland bathed in calcium-free saline containing 5 mmol EGTA the potential first went negative, as in normal saline, but instead of remaining negative the potential went slowly positive and stabilized at a value positive to the resting potential as long as LSD was perfused over the gland. If calcium was added, the potential went negative to the same value seen during treatment with LSD in normal saline. Positivity returned again if the gland was once more bathed in calcium-free saline. When a gland bathed in a medium where chloride was replaced by the larger anion isethionate was treated with LSD for 45 s the potential went slowly positive taking 1.5 min to reach maximum positivity; 6 min after removal of LSD from the perfusion medium the potential began to decrease and after 12 min the potential had returned to the resting level. In the same gland the recovery after treatment with 5-HT for the same period of time took only 4 minutes.

As was observed in the experiments where secretion was measured (Fig. 2a) the onset of the potential response to LSD was dose-dependent (Figure 7). The responses to different doses of LSD resembled those to different doses of 5-HT (Berridge & Prince, 1972b). Low doses of LSD produced oscillations similar to those produced by 5-HT, but, whereas the latter oscillations continued, those produced by LSD gradually increased until a steady plateau was reached (Figure 7b). With low doses of LSD (0.1 nM) the lag time before any response was seen was greatly prolonged (Figure 7a).

The rate of recovery of the transepithelial potential after LSD treatment can be increased by antagonists. Thus gramine reduced the recovery time of a gland treated with LSD for 1 min from 15 min to 7.5 min (Figure 8).

The effect of (+)-lysergic acid diethylamide (LSD) on the concentration of cyclic 3',5'-adenosine monophosphate (cyclic AMP)

Previously, 5-HT has been shown to cause two to four fold increases in cyclic AMP concentration in salivary glands (Prince *et al.*, 1972). A similar stimulation was also produced by LSD. In 10 experiments, the control level of cyclic AMP was 0.21 ± 0.012 pmol/gland (s.e. mean). After 10 min incubation at 27°C with either 10 nM 5-HT or 10 nM LSD this value rose to 0.31 ± 0.06 pmol/gland and 0.29 ± 0.5 pmol/gland respectively. These increases in cyclic AMP concentration were significant at the 1% level.

Discussion

The results described in this paper provide further evidence that (+)-lysergic acid diethylamide (LSD) can act on a 5-hydroxytryptamine (5-HT) receptor and suggest a possible mechanism for hallucinogenic activity. There is a close structural similarity between 5-HT and LSD (Chothia & Pauling, 1969; Kang & Green, 1970). In particular, when the indole ring of 5-HT or its derivatives is positioned over the corresponding region of LSD the positively charged nitrogen atom of both molecules coincides exactly (Kang & Green, 1970; Baker, Chothia, Pauling & Weber, 1973). Since the postively charged ethylamine side chain appears to be the active site on the 5-HT molecule (Berridge, 1972), there is reasonable justification for assuming that both molecules could activate the same receptor. Apart from this argument based on their close structural similarities, there is more direct evidence to suggest that LSD can act on the same receptor sites normally occupied by 5-HT. In salivary glands, either 5-HT or tryptamine can protect the 5-HT receptor against the action of LSD (Figure 3). Furthermore, LSD can induce all the physiological and biochemical events that have been associated with the action of 5-HT. For example, the addition of LSD caused the transepithelial potential to go negative as does 5-HT (Fig. 6) and this response is dependent on the presence of calcium and chloride. Also, the intracellular concentration of cyclic 3',5'-adenosine monophosphate (cyclic AMP) is raised by LSD as it is by 5-HT. LSD can also induce a significant increase in the concentration of cyclic AMP in rat brain (Uzunov & Weiss, 1972). These results on salivary glands suggest that LSD and 5-HT induce secretion by the same ionic mechanisms and that both stimulants combine with a common receptor.

The interaction of 5-HT and LSD at a common receptor site is also apparent from studies comparing the binding of these two molecules to synaptosomal fragments. Marchbanks (1966) found a very high affinity binding site for 5-HT which was specifically inhibited by LSD. Similarly, Farrow & Van Vunakis (1972) found a binding site on the synaptosomes from rat brain which had a high affinity for LSD. Interaction of 5.4 nM LSD with the binding site was inhibited by 70% in the presence of 1 μM 5-HT. These results are remarkably similar to those obtained with intact salivary glands where 1 μM 5-HT was able to protect the receptor from the action of 1 nM LSD (Figure 3a). Other hallucinogens (mescaline, psilocin) have been tested for their ability to inhibit LSD binding to the synaptosomal fraction and there was a remarkable correlation between their hallucinogenic potency and their ability to inhibit LSD binding (Farrow & Van Vunakis, 1972). This correlation suggests that different hallucinogens such as LSD, mescaline and psilocin may act at the same synaptic sites. Since the above hallucinogens also display cross tolerance (Brawley & Duffield, 1972) there is further reason for assuming a common site of action.

Immunological studies have also revealed that a wide range of apparently unrelated hallucinogens share many of the structural features displayed by the LSD molecule. The binding of an antigen, ^{125}I-labelled lysergimide conjugated to a protein, to its antibody is inhibited by a variety of hallucinogens and related molecules including LSD, psilocybin, psilocin, dimethyl tryptamine, 5-HT, and mescaline (Van Vunakis, Farrow, Gjika & Levine, 1971). All this evidence thus points to the 5-HT receptor as a possible site of action of all these hallucinogens. Studies on this salivary gland system add support to this notion since these glands are stimulated by a wide range of hallucinogens and related agents. Apart from LSD, the gland will respond to dimethyltryptamine, bufotenine, mescaline and 3,4-dimethoxyamphetamine (Berridge, 1972; Berridge & Prince, 1973).

The evidence discussed so far suggests that the dramatic behavioural changes induced by LSD might be caused by interference with 5-HT receptor sites in the central nervous system. Since LSD had a potent blocking effect against the action of 5-HT on the uterus it was originally proposed that its hallucinogenic properties resulted from a similar inhibitory action on central 5-HT receptors (Gaddum, 1957; Woolley & Shaw, 1957; Giarman & Freedman, 1965). Subsequent studies employing iontophoresis of LSD into different regions of the brain revealed that it can either mimic or inhibit the effect of 5-HT depending on the location and the nature of the neurones under investigation (Curtis & Davis,

1961; Boakes, Bradley, Briggs & Dray, 1970; Aghajanian, 1972a, 1972b; Tebécis & DiMaria, 1972). If hallucinogens act by mimicking some of the effects of 5-HT in the brain, it is necessary to explain why these agents induce more profound behavioural effects than the endogenous neurotransmitter (Aghajanian, 1972b).

Perhaps the very slow recovery from LSD treatment (Figs 1 and 6), which is the major difference between the action of 5-HT and LSD on isolated salivary glands, provides a clue to the hallucinogenic activity of LSD. Slow recovery after LSD treatment is not unique to salivary glands because Curtis & Davis (1961) have reported a similar phenomenon in the lateral geniculate nucleus of the cat. Here, the recovery of the orthodromic responses of neurones suppressed by the iontophoretic application of 5-HT took 5-20 s whereas recovery from inhibition induced by LSD took 10-15 minutes. The heart of the mollusc, *Venus mercenaria*, also recovers very slowly after being stimulated with LSD (Welsh & McCoy, 1957). The slow recovery after LSD treatment could be caused by the slow dispersal of a pool of LSD molecules accumulated in these tissues during the period of stimulation. In salivary glands the recovery from LSD treatment was unaffected by short treatments with inhibitors such as gramine or harmaline which caused immediate inhibition of secretion in glands stimulated with 5-HT. In the case of harmaline, there was no evidence of any competitive action even though the glands had been washed repeatedly in control medium for 30 min (Figure 4). Such observations indicate that the slow recovery may be due to LSD remaining close to the receptor rather than to LSD leaving a non-specific biophase. Further support for this view is provided by the fact that the cells can be protected from LSD by either 5-HT or tryptamine. In such a competitive situation these agonists will prevent LSD from attaching to the 5-HT receptor but should not prevent LSD from accumulating within a non-specific biophase. The biophase where LSD accumulates would thus appear to be closely associated with the 5-HT receptor. Other hallucinogens such as mescaline and dimethoxyamphetamine stimulate secretion in salivary glands and show slow recoveries after treatment (Berridge & Prince, 1973) suggesting that this latter property might be the basis of action of many hallucinogens. By contrast most other agonists, which are not hallucinogenic, show rapid recoveries (Berridge, 1972). A note of caution must be expressed when extrapolating these results to the central nervous system because bromo-LSD shows a slow recovery yet is not hallucinogenic.

The ability of the salivary gland to respond to very low concentrations of LSD (Fig. 2a) can be explained if LSD can remain attached to its receptor for long periods of time. At these low doses the frequency of LSD-receptor interactions is presumably low but since each successful interaction reverses slowly, the number of receptors occupied with LSD molecules will gradually increase with corresponding increases in the rate of secretion or change in potential. Hence the onset and peak responses to low doses of LSD should take time to develop. Such experimental results were observed (Figures 2 and 7). A similar phenomenon has been reported during stimulation of the heart of *V. mercenaria* where the threshold concentration of LSD (1×10^{-16} M) required 3 h to produce a near maximal response (Welsh & McCoy, 1957). These observations support the idea that the interaction of LSD with the receptor is very stable and reverses much more slowly than the 5-HT-receptor complex.

In the salivary gland LSD continues to activate the glands despite apparently remaining fixed to the receptor. However, the continuous occupation of the 5-HT receptor by LSD cannot account for the ability of tryptamine (Fig. 5b) or gramine (Fig. 5a) to displace LSD. The following hypothesis attempts to explain how LSD can appear to occupy a receptor yet also allow access to the same receptor site by related molecules. That part of the LSD molecule which resembles 5-HT (Chothia & Pauling, 1969; Kang & Green, 1970) is thought to interact with the receptor in the normal way. In addition to this normal interaction, the remaining regions of the LSD molecule such as the *N*-methyl group, the additional rings or the diethylamide group may also interact with regions peripheral to the normal 5-HT receptor. Since the rate of fluid secretion after stimulation with lysergic acid reverses rapidly, it is conceivable that the diethylamide group is partly responsible for this non-specific binding of LSD. Any alteration of this diethylamide group is known to reduce drastically the hallucinogenic potency of LSD (Brawley & Duffield, 1972). After a successful LSD-receptor interaction, the LSD molecule will attempt to leave the receptor in the same way as 5-HT does but the LSD molecule may remain in the vicinity of the 5-HT receptor by binding to the additional sites surrounding the receptor, perhaps by van der Waal's interactions. Without more information about the nature of the binding sites on the receptors it is impossible to say how strong, or weak, such binding would be. These peripheral binding sites may function as semi-permanent attachment points enabling the active part of the LSD molecule to flap up and down on the 5-HT receptor. The receptor will be free to react with

other molecules for brief periods while LSD is dissociated from the primary region of the 5-HT receptor and could account for the ability of tryptamine and gramine to displace LSD from the receptor. However, the ability of gramine to facilitate the release of LSD from the receptor (Fig. 5a) occurs very much more slowly than the inhibition of secretion which occurs when gramine competes with 5-HT (Figure 4). In the former case, the chance of an incoming gramine molecule attaching to the receptor is greatly reduced because it has to compete with an incumbent LSD molecule already poised above the receptor. However, when a competing molecule is successful in inserting itself onto the receptor, it might repel the LSD molecule and thus hasten its detachment from the non-specific binding sites. At the present time we cannot say how such displacement might occur. Perhaps the combination of an antagonist or another agonist causes an allosteric change in the receptor which thereby weakens the binding of LSD. When gramine competes with 5-HT both molecules have equal opportunity of reaching the receptor and at the appropriate concentration gramine induces a rapid inhibition of secretion.

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