Br. J. Pharmac. (1970), **40**, 202–218.

Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: a possible neuronal basis for the actions of LSD 25

R. J. BOAKES, P. B. BRADLEY, I. BRIGGS AND A. DRAY

Medical Research Council Neuropharmacology Unit, The Medical School, Birmingham 15

Summary

1. 5-Hydroxytryptamine (5-HT), acetylcholine (ACh), noradrenaline (NA), glutamate, D,L-homocysteic acid (DLH), glycine and γ-aminobutyric acid (GABA) were applied to single neurones in the brain stem of decerebrate cats by microiontophoresis. The abilities of D-lysergic acid diethylamide tartrate (LSD 25), methysergide maleate (UML 491) and 2-bromo-lysergic acid diethylamide (BOL 148) to antagonize the actions of these compounds were studied.

2. LSD 25 antagonized 5-HT excitation of single neurones when applied iontophoretically or administered intravenously. LSD 25 also antagonized glutamate excitation of neurones which could be excited by 5-HT. Inhibitory effects of 5-HT, the action of glutamate on neurones which could be inhibited by 5-HT and the actions of all the other compounds tested were unaffected by LSD 25.

3. Iontophoretically applied UML 491 was also a specific antagonist to 5-HT and glutamate excitation but was less potent than LSD 25, and BOL 148 rarely exhibited antagonism.

4. It is suggested that antagonism to 5-HT and glutamate excitation of brain stem neurones may be the basis of the psychotomimetic action of LSD 25. It is also suggested that there may be similarities in the mechanisms by which 5-HT and glutamate produce excitation where they act on the same neurone.

Introduction

D-Lysergic acid diethylamide (LSD 25) produces a multiplicity of pharmacologically well defined effects, most of which appear to be central in origin. The drug is best known, however, for its psychotomimetic properties and it is, at the present time, the most powerful hallucinogenic compound known to man. Considerable interest and some speculation have centred around the possible mode of action of this drug in the central nervous system.

Psychotomimetic substances, by definition, interfere with perceptual processes, and while these effects can readily be detected in man, their study in experimental animals is considerably more difficult. However, changes in the electroencephalogram of normal human subjects given small quantities of LSD 25 (Bradley, Elkes & Elkes, 1953 ; Gastaut, Ferrer, Castells, Lesevre & Lushnat, 1953) paralleled those observed in the electrocorticogram of unrestrained cats (Bradley & Elkes, 1957) and rabbits (Himwich, 1956) and the changes (increased activation) were found to be

markedly dependent on environmental influences. Other investigations in animals demonstrated decreased thresholds for sound-induced arousal responses (Bradley & Key, 1958), increased generalization of auditory stimuli (Key, 1961) and dishabituation (Key & Bradley, 1960). From the results of these and other studies (Schweigerdt, Stewart & Himwich, 1966), it was postulated that a possible site of action for LSD 25 in the brain is at the level of the brain stem reticular formation and that this action is closely related to the afferent collateral input (Bradley & Key, 1958). Furthermore, the drug appears to influence, in a highly specific manner, the neurophysiological mechanisms concerned with filtering and integration of sensory information (Key, 1965), a disturbance in the balance of which might account for disturbances in perception, and hence produce hallucinations. Investigations of this kind, whilst providing useful information of a general nature regarding the possible site of action of the drug, do not give any indication of the mechanisms involved at the neuronal level.

Gaddum (1953) demonstrated that LSD 25, in extremely low concentrations, antagonized certain peripheral actions of 5-hydroxytryptamine (5-HT). This finding led to the hypothesis (Gaddum 1954; Woolley & Shaw, 1954) that a similar antagonism to actions of 5-HT in the central nervous system might explain the psychotomimetic effects of LSD 25. So far, no conclusive evidence either in support of, or against, the " serotonin hypothesis " has been produced.

5-HT is present in the brain (Amin, Crawford & Gaddum, 1954; Twarog & Page, 1953) and the highest concentrations are in the hypothalamus and brain stem. At the subcellular level, the techniques of differential centrifugation (Michaelson & Whittaker, 1962; Zieher & De Robertis, 1963) and fluorescence histochemistry (Hillarp, Fuxe and Dahlström, 1966) have demonstrated that 5-HT is localized to presynaptic nerve terminals, and the concept of " serotoninergic " neurones has resulted. Histochemical studies of the distribution of 5-HT in the brain stem (Fuxe, 1965) have shown that this substance is present in cell bodies in the raphé nucleus and diffuse nerve terminals in the reticular formation. Furthermore, the local application of 5-HT by iontophoresis in the vicinity of neurones in the brain results in changes in neuronal activity. In those regions where the effects of 5-HT have been studied, both excitation and inhibition have been observed (Curtis & Crawford, 1969). Thus, 5-HT fulfils some of the criteria for a synaptic transmitter in the central nervous system (for review see Bradley, 1968) and an antagonism of its actions by LSD 25, if it can be demonstrated, might therefore be related to an interference with transmitter function.

Roberts & Straughan (1967), in a study of cortical neurones in the cat *encéphale isolé*, found that iontophoretically applied LSD 25 specifically antagonized the effects of 5-HT on half the neurones which were excited by this substance. However, this property was also shared by compounds which are peripheral 5-HT antagonists but which lack psychotomimetic properties—for example, 2-bromo-LSD (BOL 148).

Against the " serotonin hypothesis " is the fact that a number of derivatives of LSD 25 are more potent antagonists of 5-HT peripherally but lack psychotomimetic activity (Isbell, Miner & Logan, 1959). The classic example is BOL 148 which, even in high doses, has no psychotomimetic activity (Cerletti & Rothlin, 1955). The results of certain investigations of LSD 25/5-HT interactions in experimental animals even indicate the possibility of a synergism between some of the effects of these compounds in the central nervous system (Bradley, 1958).

The effects of LSD 25 itself, when applied iontophoretically to neurones in the brain, are mainly inhibitory (Bloom, Costa & Salmoiraghi, 1964; Bradley & Wolstencroft, 1965; Phillis & Tebēcis, 1967). Since 90% of neurones in the brain stem respond to iontophoretic application of 5-HT (Bradley & Wolstencroft, 1965), and since this region of the brain is a possible site of action of LSD 25 (Bradley & Key, 1958), it was considered that an investigation into the existence of an antagonism at the neuronal level in this region would be worthwhile. Other putative transmitters and neuronal excitants, as well as two LSD congeners, were included in order to examine the specificity of any antagonistic action found. A preliminary account of this investigation has already been published (Boakes, Bradley, Briggs & Dray, 1969) and some of the results presented at a meeting of the British Pharmacological Society (Boakes, Bradley, Briggs, and Dray, 1970).

Methods

Adult cats of both sexes were studied. The animals were decerebrated at the midcollicular level under halothane anaesthesia and the medial portion of the cerebellum was removed. One femoral vein was cannulated for a dextrose-saline drip and the intravenous injection of drugs. After withdrawal of the anaesthetic, 5-barrelled glass micropipettes (Bradley, Dhawan & Wolstencroft, 1966) of external tip diameter 6–10 μm were inserted through the exposed horizontal floor of the fourth ventricle. Penetrations were made between 2 mm and 6 mm rostral to the obex, between 2 mm either side of the midline, avoiding the midline itself and to a depth of between 1 and 6 mm. The recording barrel of the pipettes contained 4 M saline; resistances were 1·0–5 MΩ. One barrel contained 1 M saline for monitoring iontophoretic current effects, and the others drugs in aqueous solution, for iontophoretic ejection. The resistances of these barrels were between 10 and 80 MΩ. Backing currents of 15 nA were used to prevent leakage of compounds other than amino-acids. It has been found (Bradley & Candy, 1970) that there was no detectable leakage of labelled compounds into saline from micropipettes when this backing current was used. The backing currents for the amino-acids used were adjusted to the minimum necessary to prevent effects on neurone firing rates.

The method of displaying and counting neurone spikes has been described (Bradley *et al.*, 1966). Drugs were ejected iontophoretically in the vicinity of spontaneously active neurones for one or more successive 5 s epochs.

Effects of LSD 25, methysergide and BOL 148 were examined on neurones which showed consistent responses to one or more excitant or depressant compounds. The criteria used for the demonstration of antagonism to the action of a particular substance were either (*a*) reduction of the effect of that substance by at least 50% and its subsequent recovery, or (*b*) a similar reduction of that effect whilst the action of another compound remained constant. All drugs were applied iontophoretically except in those experiments where the effects of intravenous injections of LSD 25 on excitations elicited by iontophoretically applied compounds were studied.

Drugs

D-Lysergic acid diethylamide tartrate (LSD 25), methysergide maleate (UML 491) and 2-bromo-lysergic acid diethylamide (BOL 148) (Sandoz) were used in the micropipettes as 0·5% solutions in de-ionized water, except in a few preliminary experi-

ments when a 2% LSD 25 solution was used. A 0·01% solution of LSD 25 was used for intravenous injection. The following drugs were also used in the micropipettes, at the indicated concentration after adjusting the pH with sodium hydroxide or hydrochloric acid: serotonin bimaleinate, 10%, pH 4·5–6·0; monosodium glutamate, 10%, pH 8·0–9·0; D,L-homocysteic acid, 10%, pH 8·0–9·0; glycine hydrochloride, 10%, pH 3·0–4·0; γ-amino-butyric acid, 10%, pH 3·0–4·0 (Koch-Light); (−)-noradrenaline, 10%, pH 5·0–6·0 (B.D.H.); acetylcholine chloride, 10%, pH 5·0–6·0 (Hopkin & Williams).

Results

Response to 5-HT

Bradley & Wolstencroft (1965) reported that 5-HT excited some neurones and depressed others in the brain stem of unanaesthetized decerebrate cats. In the present experiments, inhibitory effects were usually weak (Fig. 2B); depression of neuronal activity appeared, in most cases, within 10 s of switching on the current, and recovery occurred within 15 s of switching off. More prolonged inhibition was only rarely seen. With repeated application of 5-HT, the inhibitory response showed no tendency to desensitization. Although no random sampling was attempted, and the selection of neurones was biased in favour of large cells with steady firing rates, the proportion inhibited by 5-HT was about 10%. Excitatory responses, on the other hand, occurred much more frequently and approximately 70% of the neurones studied were excited by 5-HT.

The excitatory response to 5-HT was usually strong and prolonged (Figs. 1B and 4A); the increase in firing rate was apparent 2 epochs (10 s) after switching on the drug, but sometimes was only seen after switching off. There was no correlation between the latency of 5-HT excitation of brain stem neurones and their initial firing rate, in contrast to the observations of Johnson, Roberts, Sobieszek & Straughan (1969). The time taken to reach a peak effect varied: the mean and S.D. for 50 units was $8{\cdot}0 \pm 2{\cdot}4$ epochs (40 ± 12 s) after switching on the current. The time taken for recovery also varied and the firing rate did not always return to its previous level (Fig. 4B). Subsequent applications of 5-HT often failed to excite neurones to the same extent as the first application. Thus, the excitatory response to 5-HT showed some desensitization but this was not as marked as the desensitization of the excitatory response of brain stem neurones to noradrenaline (Boakes, Bradley, Brookes & Wolstencroft, 1968). Occasionally biphasic responses—inhibition followed by excitation—were observed.

The proportions of neurones excited or inhibited by 5-HT in the present series of experiments are at variance with previous findings with brain stem neurones (Bradley & Wolstencroft, 1965) where 40% were found to be excited and 49% inhibited. The total number of neurones responding to application of 5-HT is, however, much the same (80–90%).

Response to iontophoretically applied LSD 25

In a few preliminary experiments, LSD 25 was applied iontophoretically from a 2% solution in the micropipette. Brief applications with this solution sometimes had markedly depressant actions on neuronal activity as previously observed by

Bradley & Wolstencroft (1964). This effect was not seen with short applications from a 0·5% solution, and therefore may have been a non-specific effect possibly related to a local anaesthetic action of the drug (Toman & Sabelli, 1968). It was often impossible to pass current through barrels containing 2% LSD 25 solution for long periods. Longer applications of LSD 25 from a 0·5% solution had a depressant action on twenty-two out of thirty-five neurones tested. This depression developed gradually, however, in contrast to the depression observed with the 2%

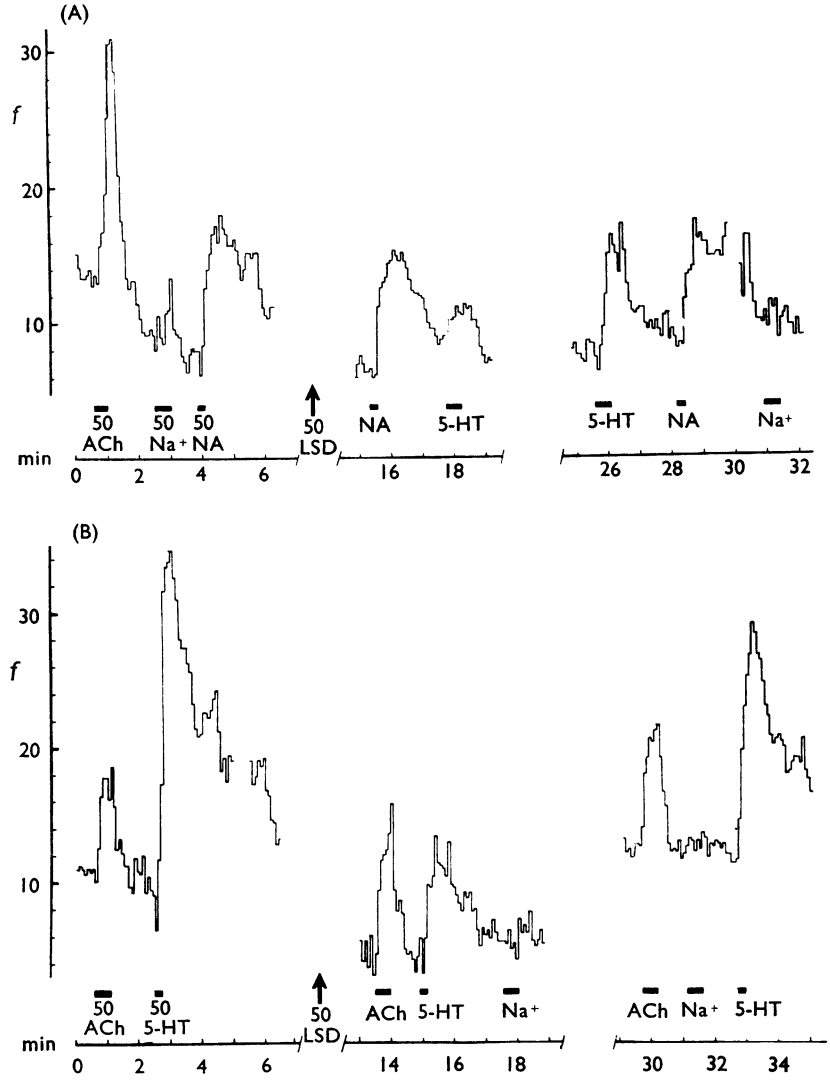

FIG. 1. Effects of iontophoretically applied LSD 25 on excitatory responses to 5-HT, NA and ACh. (A) The firing rate in impulses s^{-1} (f) of a single neurone in the brain stem of a decerebrate cat is plotted against time in min. The excitatory response to 5-hydroxytryptamine (5-HT), but not that to noradrenaline (NA), was blocked after applying D-lysergic acid diethylamide (LSD 25) for 5·5 min. A further application of 5-HT 13 min later showed partial recovery. The figures beneath the bars indicate the iontophoretic currents in nA. Current passed through a barrel of the electrode containing 1 M saline (Na^+) had no appreciable effect. (B) Application of LSD 25 for 10 min blocked the excitatory response to 5-HT but not that to acetylcholine (ACh). Partial recovery of the 5-HT response was present 18 min later.

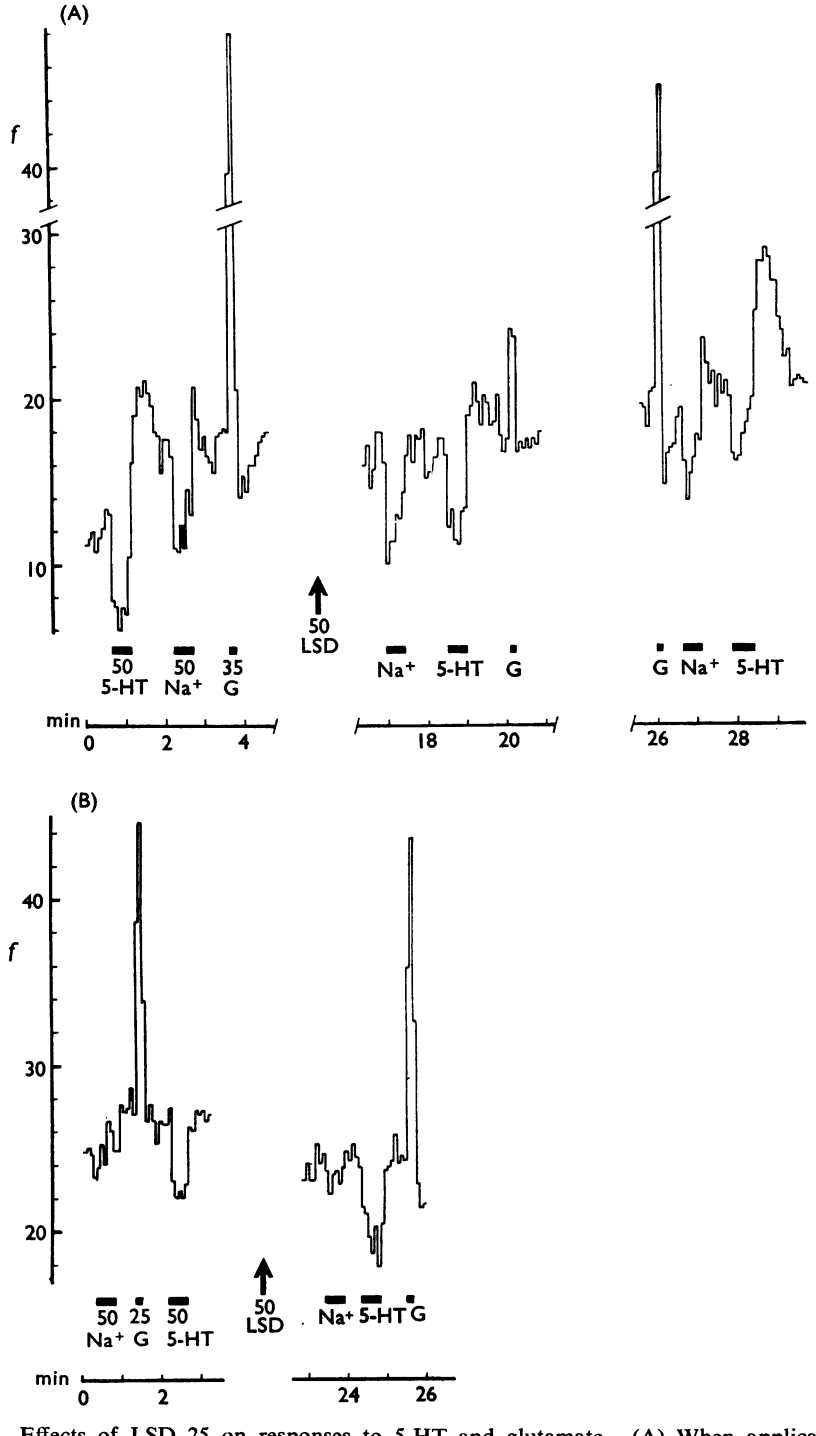

FIG. 2. Effects of LSD 25 on responses to 5-HT and glutamate. (A) When application of 5-HT caused excitation of a neurone, the responses to both 5-HT and glutamate (G) were reduced by the application of LSD 25 for 10 min. Both responses subsequently showed partial recovery. (B) When the application of 5-HT caused inhibition, LSD 25 applied for 16 min had no effect on the response to either 5-HT or glutamate. Ordinates (f): firing rate in impulses s^{-1}.

solution, and spike amplitude remained unaffected. The firing rates of five of the twenty-two neurones depressed by LSD 25 were reduced to zero. This depression was not correlated with any actions of 5-HT or noradrenaline. No excitatory effects were ever observed with iontophoretically applied LSD 25.

Responses to other compounds

The actions of iontophoretically applied acetylcholine (ACh), noradrenaline (NA), glutamate (G) and γ-aminobutyric acid (GABA) on brain stem neurones have already been described (Bradley & Wolstencroft, 1965; Bradley *et al.*, 1966; Boakes *et al.*, 1968). Both ACh and NA have depressant and excitant actions; glutamate excites most neurones and GABA inhibits most neurones. In this study, glycine (GLY) was found to be a more potent inhibitor of brain stem neurones than GABA, in agreement with the results of Hösli, Tebēcis & Filias (1969). D,L-Homocysteic acid (DLH) was also a potent excitatory compound; however, neurones excited by DLH were not always excited by glutamate and vice versa. The latency and duration of DLH excitation were longer than for glutamate.

Effect of iontophoretically applied LSD 25 on 5-HT excitation

The excitatory effects of 5-HT were suppressed, or completely blocked in some instances, by long applications of LSD 25 (Fig. 1A and B, Fig. 2A). When applied for 5 min or longer, at 50 nA, LSD 25 antagonized the excitatory actions of 5-HT on thirty-one out of thirty-two neurones. Partial recovery of 5-HT excitation was observed in twenty-one of these. Full recovery was never seen after applying LSD 25 for more than 5 min at 50 nA, and up to 3 h were then needed for partial recovery. Applications of LSD 25 at 50 nA for less than 5 min occasionally failed to antagonize 5-HT excitation. However, even when LSD 25 was ejected with low iontophoretic currents (5 or 10 nA) antagonism to 5-HT excitation could still be observed (Table 1). No evidence of potentiation of 5-HT excitation by LSD 25 was found and the effect of LSD 25, even with low currents and for short applications, was always to depress 5-HT excitation, never to increase it.

Iontophoretic application of LSD 25 was never observed to modify inhibitory actions of 5-HT (Fig. 2B).

A number of excitatory and inhibitory compounds were used to determine the specificity of the blockade of 5-HT excitation by LSD 25 (Table 2). These compounds were tested in parallel with 5-HT, and LSD 25 was routinely applied for at least 5 min at 50 nA, to effectively antagonize the 5-HT response. The excitatory actions of ACh and DLH were never affected by LSD 25 applications. The excitatory effects of NA on nine neurones were unaffected (Fig. 1A), but on one neurone excitation was depressed after application of LSD 25. However, this could have been

TABLE 1. *Numbers of 5-HT excitations antagonized by LSD 25 under different parameters*

Time of release	Current	Amount released*	Numbers of neurones	
			Blocked	Not blocked
<1 min	50 nA	<0·05 pmol	0	5
>5 min	5 nA	>0·025 pmol	4	1
>5 min	10–30 nA	>0·05–0·15 pmol	4	2
>5 min	50 nA	>0·25 pmol	31	1

* From the data of Bradley & Candy (1970).

due to desensitization. Inhibitory actions of ACh, NA, GABA and glycine were not modified by LSD 25 (Table 2).

When glutamate was used as a control, to test the specificity of the LSD 25 effects, unexpected results were obtained. LSD 25 was found to antagonize the excitatory actions of glutamate on neurones which were excitable by 5-HT (Fig. 2A), but not on neurones which were inhibited by 5-HT (Fig. 2B). LSD 25 was applied to eighteen neurones from which clear and consistent responses to both 5-HT and glutamate had been obtained: thirteen of these were excited by 5-HT and the excitatory action of glutamate was blocked by the LSD 25, concomitantly with the blocking of the 5-HT excitation; the other five were inhibited by 5-HT and the excitatory action of glutamate on these neurones was not affected by LSD 25. LSD 25 also blocked glutamate excitation of five out of seven neurones on which the action of 5-HT was not clear.

TABLE 2. *Antagonism by LSD 25, UML 491 and BOL 148 to the effects of excitatory and inhibitory agents*

	LSD 25		UML 491		BOL 148	
	Blocked	Not blocked	Blocked	Not blocked	Blocked	Not blocked
5-HT+	31	1	23	24	6	27
5-HT−	0	8	0	12	0	2
ACh+	0	15	0	9	0	9
ACh−	0	2			0	1
NA+	1	9	0	10	1	5
NA−	0	10	0	4	0	4
GLY−	0	7	0	6	1	5
GABA−	0	9	1	6	1	7
DLH+	0	8	2	9	0	5
Glutamate+	20	7	12	9	1	18

+, Excitation; −, inhibition; the numbers refer to numbers of neurones.

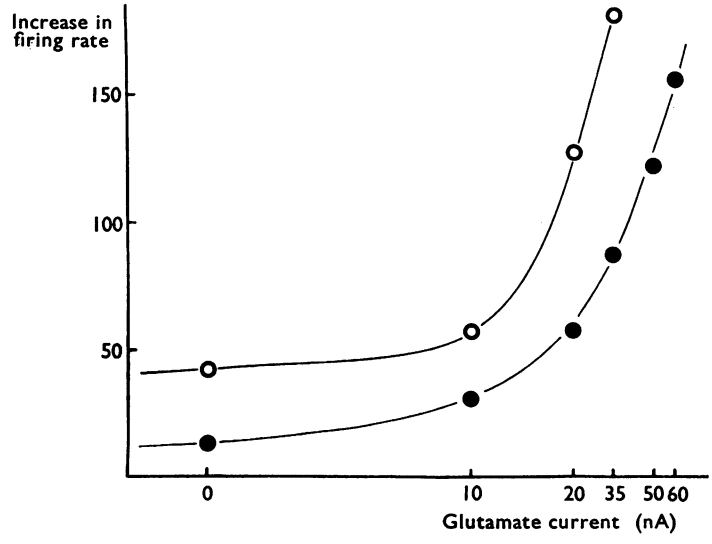

FIG. 3. Effect of LSD 25 on the graded excitatory responses of a single neurone, obtained by application of glutamate ions using different current strengths. The increase in firing rate impulses s^{-1}, plotted against glutamate current, before (○) and during (●) application of LSD 25, shows a parallel shift, suggesting that the antagonism may be competitive. The difference in the base lines may be due to antagonism by LSD 25 to the effect of glutamate leaking from the micropipette.

It was difficult to compare the time-course of the glutamate blockade with that of the 5-HT blockade, because frequent applications of 5-HT were avoided to minimize the possibility of desensitization developing. However, in some cases, the antagonism to glutamate appeared earlier than that to 5-HT, and recovery was also quicker. Attempts were made to find out whether the antagonism to glutamate excitation by LSD 25 was competitive. Blockade of the glutamate response could be overcome by increasing the current used to eject glutamate ions, but dose-response curves with upper response limits were impossible to obtain, since the firing rate increased as the glutamate current was increased and eventually individual spikes could not be differentiated. One set of results is shown in Fig. 3. Over the range of responses obtainable, the curves are parallel, suggesting that the antagonism may be competitive. No potentiation of glutamate excitation by LSD 25 was ever observed, even when the LSD 25 was applied with low currents and for short periods.

Effects of methysergide on 5-HT excitation

Methysergide was applied routinely at 50 nA for 5 min or longer. It antagonized the excitatory actions of 5-HT on twenty-three out of forty-seven neurones tested (Fig. 4A) and thus its ability to block 5-HT excitation was weaker than that of LSD 25. Furthermore, recovery after blockade by methysergide occurred more quickly and more frequently than after LSD 25. Methysergide did not affect inhibitory actions of 5-HT or any of the effects of ACh, NA, DLH, GABA and glycine (Table 2).

Methysergide was also found to antagonize the excitatory actions of glutamate on some neurones. As with LSD 25, this antagonism was present in neurones excited by 5-HT and not those inhibited by 5-HT. However, the antagonism to both glutamate and 5-HT excitation by methysergide was weaker than that of LSD 25 and glutamate excitation was more resistant to methysergide antagonism than 5-HT excitation (Table 2).

The firing rate of forty-four neurones out of sixty-three was depressed during the application of methysergide (Fig. 4A), but reduction in spike amplitude was rare.

Effects of BOL 148 on 5-HT excitation

Applications of BOL 148 at 50 nA for 5 min or more depressed 5-HT excitation in six out of thirty-three neurones examined (Fig. 4B). Thus it was a much weaker antagonist of 5-HT excitation in the brain stem than either LSD 25 or methysergide. BOL 148 blocked both 5-HT and glutamate excitation of only one neurone (Table 2). BOL 148 depressed the firing rate of twenty-eight neurones out of forty-five, but had no effect on spike amplitude.

Effects of intravenous LSD 25

An attempt was made to determine whether the blockade of 5-HT excitation by iontophoretically applied LSD 25 also occurred with systemic injection of the drug. In each of seven cats, a single neurone, excited by 5-HT, was recorded successfully during and following intravenous injection of LSD 25 (10–35 μg/kg). In five of these experiments, blockade of the action of 5-HT was seen and in another, although the neurone became silent after the injection of LSD 25, a response to

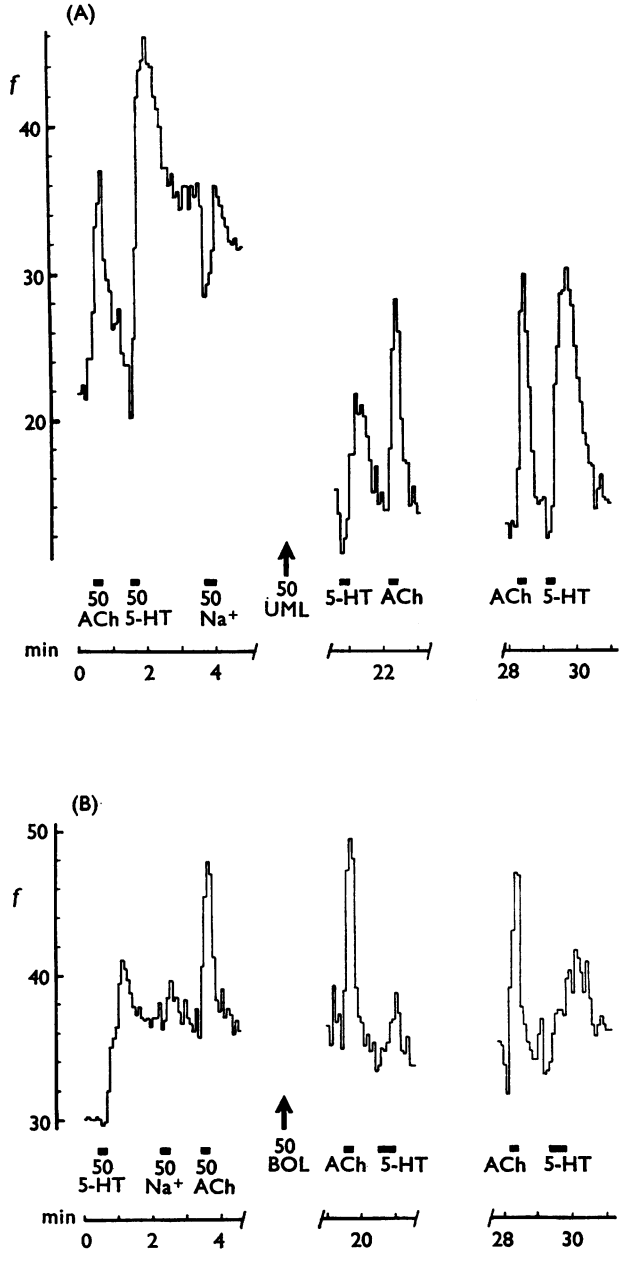

FIG. 4. A: Example of antagonism of 5-HT excitation by methysergide (UML 491). After a 10 min application of UML 491, the excitatory response to 5-HT, but not that to ACh, was blocked. Partial recovery of the 5-HT response was shown 8 min later. B: One of the few cases (six neurones) in which 2-bromo-LSD (BOL 148) blocked 5-HT excitation. After a 10 min application of BOL 148, the excitatory response to 5-HT, but not that to ACh, was blocked. Recovery was present 9 min later. Ordinates (*f*): firing rate in impulses s^{-1}.

DLH but not 5-HT could still be obtained. Two examples of the effects of intravenous LSD 25 are shown in Fig. 5. In both neurones 5-HT initially produced excitation. In Fig. 5A, the effect of 5-HT was almost completely blocked 15 min after the intravenous injection of 12 μg/kg of LSD 25 ; 40 min later the response to 5-HT showed partial recovery. In Fig. 5B, the responses to both glutamate and 5-HT were markedly depressed 5 min after 30 μg/kg LSD 25 intravenously, while the response to DLH was unaffected.

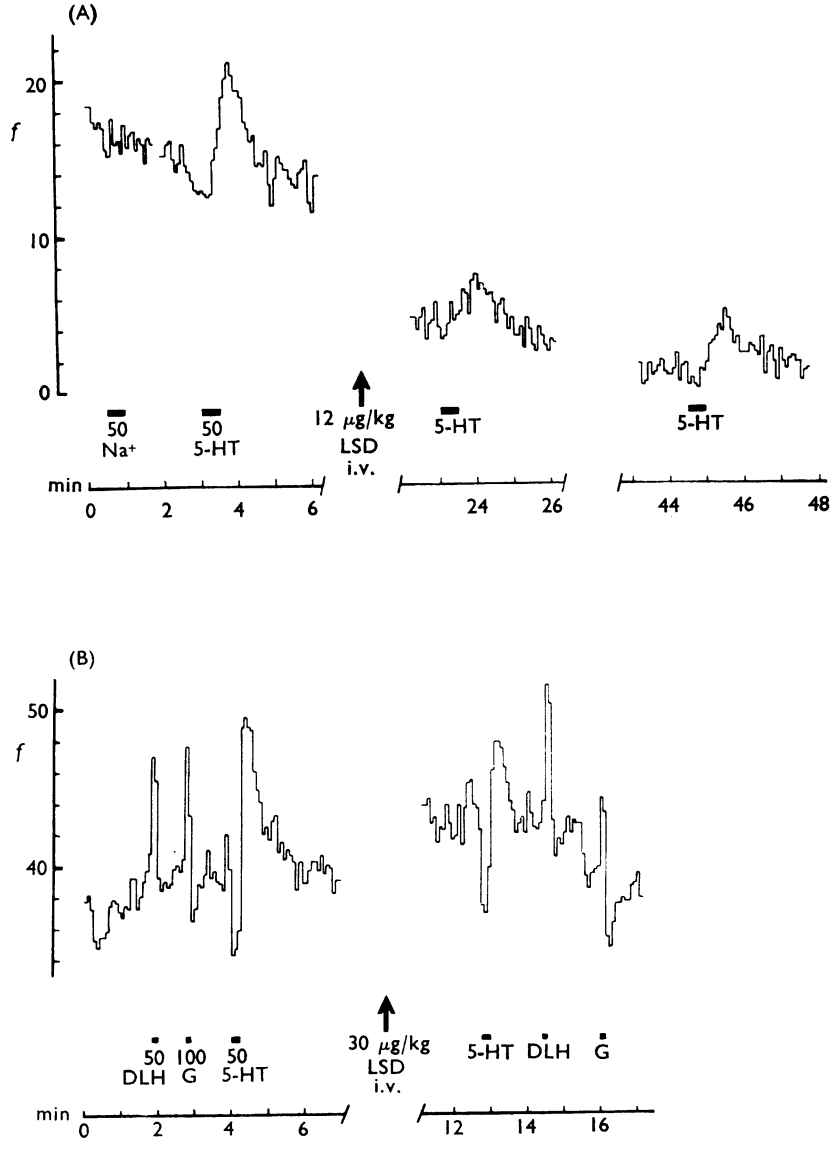

FIG. 5. Effects of intravenous injection of LSD 25 on the responses of two brain stem neurones to iontophoretic application of excitatory agents. For further details see text. Ordinates (f)=firing rate in impulses s^{-1}.

Discussion

The experimental results presented here provide direct evidence for the existence of a specific antagonism by LSD 25 to excitatory actions of 5-HT in the brain stem of the cat. In their studies of cortical neurones, Roberts & Straughan (1967) found that 5-HT could cause excitation, and that this effect was antagonized by iontophoretically applied LSD 25. However, they also found that other compounds, which do not possess hallucinogenic activity, were almost as active as LSD 25, and the proportion of 5-HT excitations which could be blocked by LSD 25 was lower than in the present experiments. In our experience, BOL 148 antagonized the excitatory effects of 5-HT in only a few cases ; methysergide showed greater activity, although considerably less than that shown by LSD 25 (Fig. 6). LSD 25 is the most powerful psychotomimetic drug known, whilst BOL 148 was found to be without hallucinogenic activity (Cerletti & Rothlin, 1955), in spite of its close structural relationship to LSD 25. Methysergide, on the other hand, has been reported to produce mental effects similar to those of LSD 25 (Abramson & Rolo, 1967 ; Bender, 1970). It would appear, therefore, that with the three derivatives of lysergic acid tested in the present study, there is a good correlation between hallucinogenic activity and potency as an antagonist to excitatory actions of 5-HT (Fig. 6). The fact that similar effects were observed in the experiments where LSD 25 was injected intravenously suggests that this drug has the same action on brain stem neurones, irrespective of whether it is applied iontophoretically or administered systemically. Thus it is possible that the antagonism to 5-HT might explain the behavioural effects of low doses of this drug. In view of the evidence concerning the site of action of LSD 25 (Bradley & Key, 1958 ; Schweigerdt *et al.*, 1966), it would seem that studies with brain stem neurones are most likely to produce data which are relevant to the mode of action of this drug.

It might be argued that the amount of LSD 25 reaching the receptor sites during iontophoretic application is higher than that attained after oral or intravenous administration. In studies of the release of labelled 5-HT and LSD 25 from micropipettes similar to those used in the present study, Bradley & Candy (1970) have determined the transport number of 5-HT as 0·17 and that of LSD 25 as 0·02. Thus

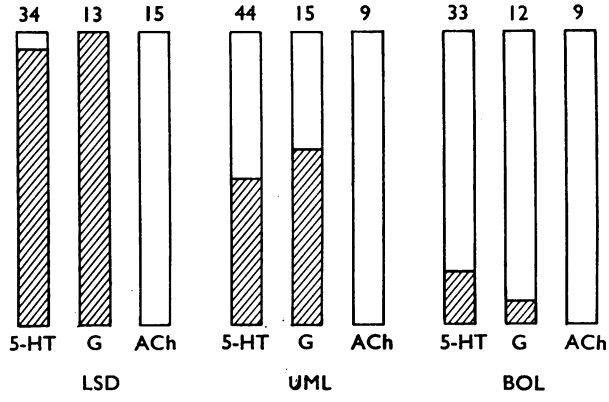

FIG. 6. Comparison of the relative activities of three lysergic acid derivatives (LSD 25, UML 491 and BOL 148), arranged in order of psychotomimetic potency, as antagonists of the excitatory actions of 5-HT, glutamate (G) and ACh. ▨, Blocked ; □, not blocked. The numbers above the columns indicate the total number of neurones in each category. Only neurones excited by 5-HT have been included.

the quantity of LSD 25 released from a micropipette during iontophoresis at 50 nA for 5 min is similar to the amount of 5-HT released during iontophoresis at 50 nA for 30 s, and the concentration of LSD 25 at a particular point will be approximately one-tenth of the concentration of 5-HT at the same point when both compounds are released by currents of 50 nA. Since the local concentration of an ion released from a point source is independent of the duration of release if this is long (Curtis, Perrin & Watkins, 1960), it would appear that the LSD 25 concentration must be maintained for at least 1 min to enable the drug to have an effect (Table 1). Using the transport number for LSD 25 calculated by Bradley & Candy (1970), and the equations derived by Curtis *et al.* (1960), the concentrations of LSD 25 at distances of 50 μm and 100 μm from the tip of the micropipette may be calculated, assuming free distribution of the drug. These concentrations are $8{\cdot}8 \times 10^{-6}$M and $6{\cdot}2 \times 10^{-6}$M respectively, expelled at 50 nA, and $8{\cdot}8 \times 10^{-7}$M and $6{\cdot}2 \times 10^{-7}$M at 5 nA, both currents being effective in producing antagonism (Table 1). These values are comparable with the tissue concentration of $2{\cdot}2 \times 10^{-8}$M LSD 25 found in the cat brain stem after the intravenous injection of 25 μg/kg LSD 25 (Bradley & Candy, 1970).

Kawai & Yamamoto (1969) found that 10^{-7} to 10^{-6}M LSD 25 antagonized the effect of 5-HT on surface potentials evoked in slices of guinea-pig superior colliculus. These concentrations are similar to those obtained in our experiments during iontophoresis. Kawai & Yamamoto also suggested that 1×10^{-8}M LSD 25 facilitated the effect of 5-HT. It seems possible that the effect observed by these workers was not facilitation but summation of the effects of the two substances. In the present experiments, no potentiation of the effects of 5-HT by LSD 25 was observed even after short periods of iontophoretic application of LSD 25, or after intravenous injection of LSD 25 giving tissue concentrations of about 10^{-8}M.

Biochemical studies have demonstrated small increases in 5-HT levels in brain following administration of LSD 25 (Freedman & Giarman, 1962) and a concomitant lowering of the 5-HT metabolite, 5-hydroxyindoleacetic acid (Rosecrans, Lovell & Freedman, 1967). LSD 25 has also been found to reduce the turnover rate of 5-HT (Andén, Corrodi, Fuxe & Hökfelt, 1968 ; Costa, 1969) and Andén *et al.* attributed this effect to a feedback inhibition of serotoninergic neurones, following stimulation of 5-HT receptors by LSD 25. This suggestion, however, is not supported by our results. It seems more likely that LSD 25 may have a direct action on serotoninergic neurones, preventing the release of 5-HT (Chase, Breese & Kopin, 1967 ; Diaz, Ngai & Costa, 1968 ; Katz & Kopin, 1969 ; Aghajanian, 1970). LSD 25 has also been reported to enhance binding in 5-HT-containing granules (Giarman & Freedman, 1965). These findings are not incompatible with our own observations.

The idea that LSD 25 might mimic the actions of 5-HT in the central nervous system is based largely on indirect evidence. Although the principal action of LSD 25 at peripheral receptors is to antagonize 5-HT, it can, in some instances, mimic actions of 5-HT (Shaw & Woolley, 1956). It has been reported (Mantegazzini, 1966) that the administration of the 5-HT precursor, 5-HTP, which produces an increase in 5-HT levels in the brain, can cause excitement in animals resembling some effects of LSD 25. However, small doses of 5-HTP produce sedation and only when this substance is administered either in large doses, or in conjunction with monoamine oxidase inhibitors, is behavioural excitation observed. The excitatory effects of 5-HTP have recently been attributed, not to the 5-HT formed, but to a release of noradrenaline (Brodie, Comer, Costa & Dlabac, 1966), or to the

actions of bufotenine formed in the brain (Mandell & Spooner, 1969). Most evidence now seems to favour a depressant role for 5-HT in the central nervous system (Brodie & Reid, 1968 ; Takagi, Satoh, Yamatsu, Kimura & Nakama, 1968) and it is possible that this substance may be involved in the physiological mechanisms of sleep (Koella, 1968 ; Jouvet, 1969).

LSD 25 has also been found to have similar effects to 5-HT, or to facilitate actions of 5-HT, when applied iontophoretically (Curtis & Davis, 1962 ; Krnjević & Phillis, 1963 ; Phillis, Tebēcis & York, 1967), but these observations are obscured by the use of barbiturate anaesthetized preparations. It has been demonstrated that the magnitude and frequency of occurrence of excitation by monoamines are reduced by barbiturates (Satinsky, 1967 ; Johnson, Roberts & Straughan, 1969). This may account for the failure in many instances to find excitatory actions of 5-HT in regions where such actions have subsequently been demonstrated (Bradley & Wolstencroft, 1965 ; Satinsky, 1967 ; Roberts & Straughan, 1967). Thus, an antagonism between LSD 25 and 5-HT would not have been observed in the early experiments in which excitation by 5-HT was not found. It is possible that the depressant action of LSD 25 on some neurones (Bradley & Wolstencroft, 1964) is not a 5-HT-mimicking action, but is due to a local anaesthetic effect of the drug (Toman & Sabelli, 1968).

Sheard & Aghajanian (1969) found that electrical stimulation of 5-HT-containing neurones of the midbrain raphé nucleus of the rat, which caused release of 5-HT, also resulted in a failure of the normal process of habituation to sensory stimuli and a rise in body temperature, effects resembling those seen after LSD 25 administration (Key & Bradley, 1960 ; Key, 1965). These findings appear to support the suggestion that LSD 25 mimics the actions of 5-HT in the central nervous system and it is difficult to reconcile them with our results. However, Kostowski, Giacalone, Garattini & Valzelli (1969) have observed e.e.g. " sleep patterns " and behavioural calmness during stimulation of the midbrain raphé of rats. These effects are opposite to those seen after LSD 25 administration and support the idea that LSD 25 antagonizes the physiological actions of 5-HT. The difference between the two sets of results is still to be resolved.

It seems unlikely that LSD 25 acts by mimicking the actions of noradrenaline in the brain stem, since in the present experiments it did not excite neurones which were excited by NA, nor did it inhibit in any consistent way those neurones which were inhibited by NA.

Finally, the somewhat unexpected finding that LSD 25 antagonized excitatory actions of glutamate only where it also antagonized excitation by 5-HT deserves some further comment. This is the first documented example of a specific antagonism to glutamate excitation. It is made more striking by the fact that DLH excitation was unaffected by LSD 25. A reduction of glutamate excitation by LSD 25 (Krnjević & Phillis, 1963 ; Phillis *et al.*, 1967) and by ergometrine (Biscoe & Straughan, 1966) has been reported, but these workers did not study the specificity of this antagonism. It is of interest that Roberts & Straughan (1967) found that the proportion of neurones excited by 5-HT was greater when glutamate was used to excite the neurones, in comparison with spontaneously active neurones. It should also be noted that in 1954, Hoff & Arnold reported that glutamic acid or succinic acid (which can give rise to glutamate via the tricarboxylic acid cycle), given orally or intravenously, was able to interrupt or retard the mental effects of LSD 25 and,

further, that glutamic acid can inhibit the rise in body temperature induced by LSD 25 (Friedhoff & Abrams, 1960).

A connexion between the excitatory actions of glutamate and of 5-HT is difficult to account for, especially as glutamate can excite many neurones which are either unaffected or inhibited by 5-HT. It might be speculated that 5-HT excitation is produced by a mechanism which can also be activated by glutamate probably via a different receptor. The presence of 5-HT receptors allows LSD 25 to inactivate this mechanism, possibly by steric occlusion if the two receptors are adjacent, or by some form of allosteric inactivation.

Because LSD 25 is specifically antagonistic to 5-HT excitation and not to 5-HT inhibition, it might be postulated that excitation and inhibition by 5-HT are mediated by different types of receptors, if in fact inhibitory effects are due to an action on specific receptors. Our experiments demonstrate that the pharmacology of 5-HT receptors in the brain stem is not the same as that at the periphery, because the relative potencies of the three derivatives of lysergic acid studied differed at these two sites. The suggestion (Isbell *et al.*, 1959) that psychotomimetic activity is not due to antagonism to 5-HT, because the peripheral anti-5-HT potencies of these compounds bear no relationship to hallucinogenic potencies, now seems to be invalidated. It is notable that, although some potent peripheral 5-HT antagonists are not hallucinogenic, no hallucinogens of the indole or lysergic acid series are without peripheral anti-5-HT activity. It remains to be seen whether any other chemically different psychotomimetics are able to modify the actions of 5-HT in the brain.

REFERENCES

ABRAMSON, H. A. & ROLO, A. (1967). Comparison of LSD with methysergide and psilocybin on test subjects. In *The Use of LSD in Psychotherapy and Alcoholism*, ed. Abramson, H. A., pp. 53–73. New York: Bobbs-Merrill Co. Inc.

AGHAJANIAN, G. K. (1970). LSD and mescaline: comparison of effects on single units in the midbrain raphé. (Discussion.) In *Psychotomimetic Drugs*, ed. Efron, D. H., p. 173. New York: Raven Press.

AMIN, A. H., CRAWFORD, T. B. B. & GADDUM, J. H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol., Lond.*, **126**, 596–618.

ANDÉN, N.-E., CORRODI, H., FUXE, K. & HÖKFELT, T. (1968). Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. *Br. J. Pharmac.*, **34**, 1–7.

BENDER, L. (1970). Children's reactions to psychotomimetic drugs. (Discussion.) In *Psychotomimetic Drugs*, ed. Efron, D. H., p. 272. New York: Raven Press.

BISCOE, T. J. & STRAUGHAN, D. W. (1966). Micro-electrophoretic studies of neurones in the cat hippocampus. *J. Physiol., Lond.*, **183**, 341–359.

BLOOM, F. E., COSTA, E. & SALMOIRAGHI, G. C. (1964). Analysis of individual rabbit olfactory bulb neuron responses to microelectrophoresis of acetylcholine, noreprinephrine and serotonin synergists and antagonists. *J. Pharmac. exp. Ther.*, **146**, 16–23.

BOAKES, R. J., BRADLEY, P. B., BRIGGS, I. & DRAY, A. (1969). Antagonism by LSD to effects of 5-HT on single neurones. *Brain Res.*, **15**, 529–531.

BOAKES, R. J., BRADLEY, P. B., BRIGGS, I. & DRAY, A. (1970). Effects of lysergic acid derivatives on 5-hydroxytryptamine excitation of brain stem neurones. *Br. J. Pharmac.*, **38**, 453*P*.

BOAKES, R. J., BRADLEY, P. B., BROOKES, N. & WOLSTENCROFT, J. H. (1968). Characteristics of the response of brain-stem neurones to noradrenaline. *Br. J. Pharmac. Chemother*, **32**, 417*P*.

BRADLEY, P. B. (1958). The effects of 5-hydroxytryptamine on the electrical activity of the brain an on behaviour in the conscious cat. In *5-Hydroxytryptamine*, ed. Lewis, G. P., pp. 214–220 London: Pergamon Press.

BRADLEY, P. B. (1968). Synaptic transmission in the central nervous system and its relevance for drug action. *Int. Rev. Neurobiol.*, **11**, 1–56.

BRADLEY, P. B. & CANDY, J. M. (1970). Iontophoretic release of acetylcholine, noradrenaline, 5-hydroxytryptamine and D-lysergic acid diethylamide from micropipettes. *Br. J. Pharmac.*, **40**, 194–201.

BRADLEY, P. B., DHAWAN, B. N. & WOLSTENCROFT, J. H. (1966). Pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. *J. Physiol., Lond.*, **183**, 658–674.

BRADLEY, P. B., ELKES, C. & ELKES, J. (1953). On some effects of lysergic acid diethylamide (LSD 25) in normal volunteers. *J. Physiol., Lond.*, **121**, 50–51*P*.

BRADLEY, P. B. & ELKES, J. (1957). The effects of some drugs on the electrical activity of the brain. *Brain*, **80**, 77–117.

BRADLEY, P. B. & KEY, B. J. (1958). The effect of drugs on arousal responses produced by electrical stimulation of the reticular formation of the brain. *EEG Clin. Neurophysiol.*, **10**, 97–110.

BRADLEY, P. B. & WOLSTENCROFT, J. H. (1964). The action of drugs on single neurones in the brain stem. In *Neuropsychopharmacology*, vol. 3, ed. Bradley, P. B., Flügel, F. and Hoch, P., pp. 237–240. Amsterdam: Elsevier.

BRADLEY, P. B. & WOLSTENCROFT, J. H. (1965). Actions of drugs on single neurones in the brainstem. *Br. med. Bull.*, **21**, 15–18.

BRODIE, B. B., COMER, M. S., COSTA, E. & DLABAC, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.*, **152**, 340–349.

BRODIE, B. B. & REID, W. D. (1968). Serotonin in brain: functional considerations. *Adv. Pharmac.*, **6B**, 97–113.

CERLETTI, A. & ROTHLIN, E. (1955). Role of 5-hydroxytryptamine in mental diseases and its antagonism to lysergic acid derivatives. *Nature, Lond.*, **176**, 785–786.

CHASE, T. N., BREESE, G. R. & KOPIN, I. J. (1967). Serotonin release from brain slices by electrical stimulation: regional differences and effect of LSD. *Science, N.Y.*, **157**, 1461–1463.

COSTA, E. (1969). Turnover rate of neuronal monoamines: pharmacological implications. In *The Present Status of Psychotropic Drugs*, ed. Cerletti, A. and Bové, F. J., pp. 11–35. Amsterdam: Excerpta Medica Foundation.

CURTIS, D. R. & CRAWFORD, J. M. (1969). Central synaptic transmission—microelectrophoretic studies. *Ann. Rev. Pharmac.*, **9**, 209–240.

CURTIS, D. R. & DAVIS, R. (1962). Pharmacological studies upon neurones of the lateral geniculate nucleus of the cat. *Br. J. Pharmac. Chemother.*, **18**, 217–246.

CURTIS, D. R., PERRIN, D. D. & WATKINS, J. C. (1960). The excitation of spinal neurones by ionophoretic application of agents which chelate calcium. *J. Neurochem.*, **6**, 1–20.

DIAZ, P. M., NGAI, S. H. & COSTA, E. (1968). Factors modulating brain serotonin turnover. *Adv. Pharmac.*, **6B**, 75–92.

FREEDMAN, D. X. & GIARMAN, N. J. (1962). LSD-25 and the status and level of brain serotonin. *Ann. N.Y. Acad. Sci.*, **96**, 98–106.

FRIEDHOFF, A. J. & ABRAMS, B. (1960). Effect of glutamic acid and glutamine on the pyretogenic action of lysergic acid diethylamide. *J. clin. exp. Psychopathol.*, **21**, 7–10.

FUXE, K. (1965). Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.*, **64**, suppl. 247, 37–85.

GADDUM, J. H. (1953). Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. *J. Physiol., Lond.*, **121**, 15*P*.

GADDUM, J. H. (1954). Drugs antagonistic to 5-hydroxytryptamine. In *Ciba Symposium on Hypertension: Humoral and Neurogenic Factors*, ed. Wolstenholme, G. E. W. and Cameron, M. P., pp. 75–77. London: J. & A. Churchill Ltd.

GASTAUT, H., FERRER, S., CASTELLS, C., LESEVRE, N. & LUSHNAT, K. (1953). Action de la diéthylamide de l'acid d-lysergique (LSD 25) sur les fonctions psychiques et l'électroencéphalogramme. *Confinia Neurol.*, **13**, 102–120.

GIARMAN, N. J. & FREEDMAN, D. X. (1965). Biochemical aspects of the actions of psychotomimetic drugs. *Pharmac. Rev.*, **17**, 1–25.

HILLARP, N.-Å., FUXE, K. & DAHLSTRÖM, A. (1966). Demonstration and mapping of central neurons containing dopamine, noradrenaline, and 5-hydroxytryptamine and their reactions to psychopharmaca. *Pharmac. Rev.*, **18**, 727–741.

HIMWICH, H. E. (1956). The effect of Frenquel on EEG changes produced by LSD-25 and mescaline. In *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry*, ed. Cholden, L., pp. 19–26. London: Grune & Stratton.

HOFF, H. & ARNOLD, O. H. (1954). Die Therapie der Schizophrenie. *Wien. Klin. Wschr.*, **66**, 345–352.

HÖSLI, L., TEBĒCIS, A. K. & FILIAS, N. (1969). Effects of glycine, beta-alanine and GABA, and their interaction with strychnine, on brain stem neurones. *Brain Res.*, **16**, 293–295.

ISBELL, H., MINER, E. J. & LOGAN, C. R. (1959). Relationships of psychotomimetic to anti-serotonin potencies of congeners of lysergic acid diethylamide (LSD 25). *Psychopharmacologia*, **1**, 20–28.

JOHNSON, E. S., ROBERTS, M. H. T. & STRAUGHAN, D. W. (1969). The responses of cortical neurones to monoamines under differing anaesthetic conditions. *J. Physiol., Lond.*, **203**, 261–280.

JOHNSON, E. S., ROBERTS, M. H. T., SOBIESZEK, A. & STRAUGHAN, D. W. (1969). Noradrenaline sensitive cells in cat cerebral cortex. *Int. J. Neuropharmac.*, **8**, 549–566.

JOUVET, M. (1969). Biogenic amines and the states of sleep. *Science, N.Y.*, **163**, 32–41.

KATZ, R. I. & KOPIN, I. J. (1969). Effect of D-LSD and related compounds on release of norepinephrine-H^3 and serotonin-H^3 evoked from brain slices by electrical stimulation. *Pharmac. Res. Comm.*, **1**, 54–62.

KAWAI, N. & YAMAMOTO, C. (1969). Effects of 5-hydroxytryptamine, LSD and related compounds on electrical activities evoked *in vitro* in thin sections from the superior colliculus. *Int. J. Neuropharmac.*, **8**, 437–449.

KEY, B. J. (1961). The effect of drugs on discrimination and sensory generalisation of auditory stimuli in cats. *Psychopharmacologia*, **2**, 352–363.

KEY, B. J. (1965). Effect of lysergic acid diethylamide on potentials evoked in specific sensory pathways. *Br. med. Bull.*, **21**, 30–35.

KEY, B. J. & BRADLEY, P. B. (1960). The effects of drugs on conditioning and habituation to arousal stimuli in animals. *Psychopharmacologia*, **1**, 450–462.

KOELLA, W. P. (1968). Serotonin and sleep. In *Psychopharmacology: A Review of Progress 1957–1967*, ed. Efron, D. H., pp. 541–543. Washington, D.C.: U.S. Government Printing Office.

KOSTOWSKI, W., GIACALONE, E., GARATTINI, S. & VALZELLI, L. (1969). Electrical stimulation of midbrain raphé: biochemical, behavioral and bioelectrical effects. *Eur. J. Pharmacol.*, **7**, 170–175.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Actions of certain amines on cerebral cortical neurones. *Br. J. Pharmac. Chemother.*, **20**, 471–490.

MANDELL, A. J. & SPOONER, C. E. (1969). An N,N-indole transmethylation theory of the mechanism of MAOI-indole amino acid load behavioural activation. In *Schizophrenia, Current Concepts and Research*, ed. Siva Sankar, D. V., pp. 496–505. Hicksville, N.Y.: P.J.D. Publications.

MANTEGAZZINI, P. (1966). Pharmacological actions of indolealkylamines and precursor aminoacids on the central nervous system. In *Handbook of Experimental Pharmacology*, vol. 19, 5-Hydroxytryptamine and related indolealkylamines, ed. Erspamer, V., pp. 424–470. Berlin: Springer Verlag.

MICHAELSON, I. A. & WHITTAKER, V. P. (1962). The distribution of hydroxytryptamine in brain fractions. *Biochem. Pharmac.*, **11**, 505–506.

PHILLIS, J. W. & TEBĒCIS, A. K. (1967). The responses of thalamic neurones to iontophoretically applied monoamines. *J. Physiol., Lond.*, **192**, 715–745.

PHILLIS, J. W., TEBĒCIS, A. K. & YORK, D. H. (1967). The inhibitory action of monoamines on lateral geniculate neurones. *J. Physiol., Lond.*, **190**, 563–581.

ROBERTS, M. H. T. & STRAUGHAN, D. W. (1967). Excitation and depression of cortical neurones by 5-hydroxytryptamine. *J. Physiol., Lond.*, **193**, 269–294.

ROSECRANS, J. A., LOVELL, R. A. & FREEDMAN, D. X. (1967). Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine. *Biochem. Pharmac.*, **16**, 2011–2021.

SATINSKY, D. (1967). Pharmacological responsiveness of lateral geniculate nucleus neurones. *Int. J. Neuropharmac.*, **6**, 387–397.

SCHWEIGERDT, A. K., STEWART, A. H. & HIMWICH, H. E. (1966). An electrographic study of d-lysergic acid diethylamide and nine congeners. *J. Pharmac. exp. Ther.*, **151**, 353–359.

SHAW, E. & WOOLLEY, D. W. (1956). Some serotonin like activities of lysergic acid diethylamide. *Science, N.Y.*, **124**, 121–122.

SHEARD, M. H. & AGHAJANIAN, G. K. (1969). Neural release of serotonin: interaction with drugs. In *The Present Status of Psychotropic Drugs*, ed. Cerletti, A. and Bové, F. J., pp. 323–324. Amsterdam: Excerpta Medica Foundation.

TAKAGI, H., SATOH, M., YAMATSU, K., KIMURA, K. & NAKAMA, M. (1968). Central effects of 3,4-dihydroxyphenylalanine and 5-hydroxytryptophane on tetrabenazine pretreated rabbits with special reference to the possible role of catecholamine and serotonin in the brain. *Int. J. Neuropharmac.*, **7**, 265–273.

TOMAN, J. E. P. & SABELLI, H. C. (1968). Neuropharmacology of earthworm giant fibres. *Int. J. Neuropharmac.*, **7**, 543–556.

TWAROG, B. M. & PAGE, I. H. (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.*, **175**, 157–161.

WOOLLEY, D. W. & SHAW, E. (1954). A biochemical and pharmacological suggestion about certain mental disorders. *Science, N.Y.*, **119**, 587–588.

ZIEHER, L. M. & DE ROBERTIS, E. (1963). Subcellular localization of 5-hydroxytryptamine in rat brain. *Biochem. Pharmac.*, **12**, 596–598.

(*Received May* 26, 1970)