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Action of LSD on raphe neurons and effect on presumed serotonergic raphe synapses

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Work on the pharmacology of lysergic acid diethylamide (LSD) has led to postulates that LSD decreases release of serotonin (5-HT), from presynaptic sites[8] stimulates postsynaptic 5-HT receptors[2,3], and also blocks the effect of 5-HT at the postsynaptic membrane[5,6,22]. Boakes *et al.*[5] and Roberts and Straughan[22] demonstrated that LSD can block the excitation produced by 5-HT when applied by iontophoresis to brain stem and cortical neurons, but in these studies, there was no attempt to correlate this effect with a synaptic input. From these studies, it is uncertain whether LSD blocked synaptic or non-synaptic effects of 5-HT on the neurons.

In previous work, an excitatory serotonergic synapse originating in the nucleus paragigantocellularis lateralis (NPL) of the medulla (corresponding to group B-3 of Dahlström and Fuxe[13]) and terminating on pontine and midbrain raphe neurons was postulated[9,10]. This postulate was formulated from demonstration of excitation by iontophoresed 5-HT on 97 of 100 raphe neurons which were also excited synaptically by NPL stimulation. An inhibitory 5-HT synapse was also postulated in another group of 25 raphe neurons that were inhibited by NPL stimulation, of which 20 were also inhibited by iontophoresed 5-HT[9,10].

In the present work LSD was tested as a serotonin blocking agent on the postulated excitatory and inhibitory serotonergic raphe synapses described above, and on other raphe neurons not responding to NPL stimulation. The results show that LSD can block the postulated excitatory 5-HT synapse but not the inhibitory one.

The methods were essentially the same as described previously[9]. Sprague–Dawley rats were anesthetized with 30 mg/kg pentobarbital, and after placement in a stereotaxic apparatus, a midline cerebellectomy was done to expose the floor of the fourth ventricle. A glass coated tungsten stimulating electrode was placed stereotaxically in the NPL (see ref. 9). A 5-barrel micropipette (see below), inclined 30° posteriorly, was placed under visual control on the midline of the fourth ventricle. Recordings were taken between 3 and 6 mm anterior to the obex at depths of 0.5–3.5 mm.

Five-barrel micropipettes were used for recording unit action potentials and

iontophoresis of drugs. The 5-barrel micropipettes were prepared in the fashion described by Salmoiraghi and Weight[24] and the side barrels filled with serotonin creatinine sulfate (5×10^{-2} *M*, pH 5), lysergic acid diethylamide bitartrate (3×10^{-4} *M* in saline, pH 3.5) and DL-homocysteic acid (DLH, 0.25 *M*, pH 8–9). The fourth side barrel was filled with 3 *M* NaCl and used for electrical balance[24]. The center barrel was filled with 2 *M* NaCl and 0.5 *M* Fast green FCF and was used for recording extracellular potentials and for marking tip location after completion of the experiment (see ref. 9). Standard iontophoretic techniques were employed to eject 5-HT as a cation and DLH as an anion[24]. LSD was combined with NaCl to facilitate its passage from the micropipette[11].

Standard electrophysiological recording techniques were employed. Both direct film records and poststimulus time histograms computed with a computer of average transients (CAT 1000) were employed to demonstrate synaptic response.

The possibility of synaptic block was investigated by testing synaptic efficacy before and after LSD. Repetitive stimulation of the synapse was done to establish threshold, and twice threshold stimulus strengths and then film records of 10–20 consecutive responses to threshold and twice threshold stimuli were taken. For the excitatory synapses the number of failed excitations at threshold were counted before and after LSD application. Poststimulus time histograms summating responses to 50 consecutive stimuli before and after LSD application were also employed to demonstrate LSD blockade in some cases. For the inhibitory synapse, poststimulus time histograms of inhibitory response before and after LSD demonstrated the presence or absence of blockade.

After completion of the experiments brains were fixed in 10% formalin and cut on a freezing microtome. Identification of the Fast green spots marking recording sites was carried out as described previously.

The LSD for intravenous (i.v.) use was made up in a solution of saline at a strength of 10 μg/ml. The usual test dose was 37 μg/kg but occasionally doses of 75 or 150 μg/kg were employed.

As in the previous work, neurons were classified by their response to NPL stimulation as synaptically driven (D) cells, inhibited (I) cells, or non-responsive (NR) cells[9].

In the present experiments, effects of LSD and 5-HT were tested on 53 spontaneously firing units in 19 animals. Sixteen units were tested with LSD by both iontophoretic and intravenous routes, 32 units were tested with LSD by the iontophoretic route only and 5 units tested with LSD by the intravenous route only.

In the experimental protocol, neurons from which recordings were taken were classified as D, I or NR cells by their response to NPL stimulation and then the effect of 5-HT and LSD tested on spontaneous activity. Twenty-six D cells were studied of which 24 were excited by 5-HT and 2 inhibited. Nine I cells were studied of which 2 were excited by 5-HT and 7 inhibited. Eighteen NR cells were studied of which 8 were excited by 5-HT and 10 inhibited.

Excitatory responses to 5-HT were monophasic (*i.e.*, simple excitation or inhibition) in a majority of cases, but biphasic responses with initial inhibition and

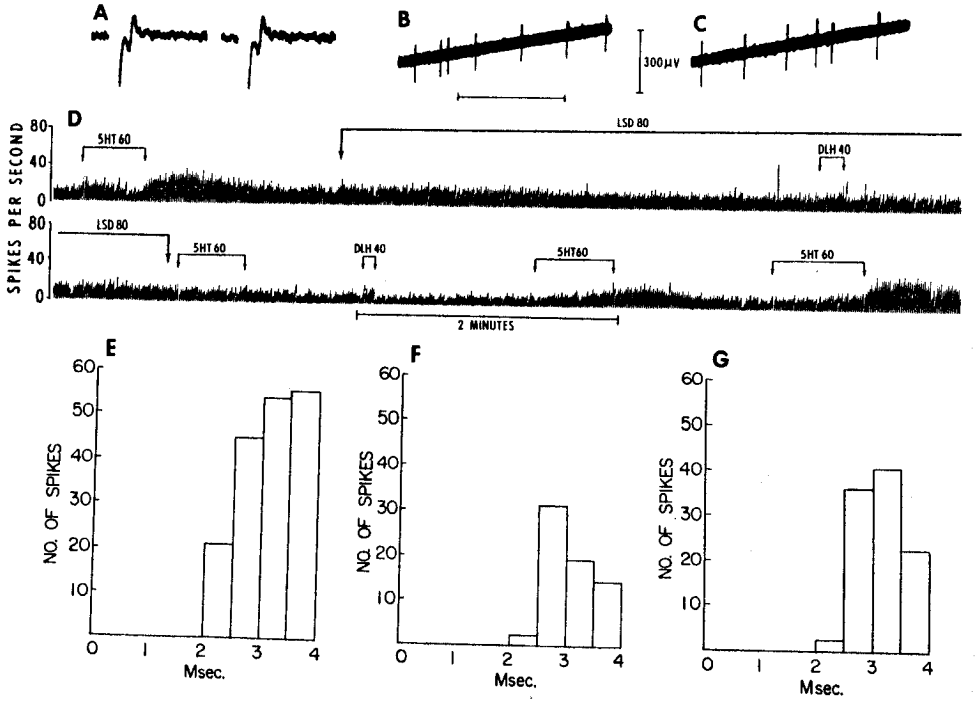

Fig. 1. Blockade of effect of iontophoresed 5-HT and partial block of serotonergic synapse on a raphe D cell by LSD. A: oscilloscope recording of stimulus response sequence to two consecutive stimuli 2 sec apart (0.9 V, 1.0 msec), stimulus delivered to NPL at the break in the oscilloscope trace. B and C: running oscilloscope records of action potential during (B) and after (C) LSD application by iontophoresis. Horizontal bar represents 10 msec for A and 50 msec for B and C. D: analog records from integrator of cell firing frequency demonstrating effect of drug application. Each spike is proportional to the number of firings of the neuron per minute. Note excitatory response to 5-HT before LSD, no response to 5-HT immediately after LSD, and gradual return of responsiveness to 5-HT over next 5 min. DLH (DL-homocysteic acid) is applied during and after LSD to demonstrate excitability of the cell. Note also, the neuron fires continuously throughout the application of LSD although the rate diminishes somewhat. E, F and G: poststimulus time histograms reconstructed from computer of average transients records demonstrating efficacy of threshold stimulus (0.45 V, 1.0 msec) delivered to NPL. E, before, F, during, and G, 5 min after LSD application. There is partial block of synaptic effect with slow but partial recovery.

later excitation were seen in 10 units (Fig. 1). Similar responses to 5-HT were described by Boakes *et al.* for neurons in the reticular formation[5] and by Roberts and Straughan for neurons in the cortex[22].

In most cases the response to 5-HT was relatively slow in developing (10–30 sec). Other authors have observed similar slowly developing responses. In the reports of Boakes *et al.*[5] and Roberts and Straughan[22], response latencies of 15–25 sec between initiation of iontophoresis and onset of 5-HT effect can be seen. The long response latencies may relate to the low transport number of 5-HT when compared to other substances such as norepinephrine[18].

Table I summarizes the data on the 48 neurons to which LSD was applied iontophoretically and 21 neurons for which LSD was given i.v. (including 16 units

TABLE I

EFFECT OF 5-HT AND LSD ON SPONTANEOUS ACTIVITY OF D, I AND NR CELLS AND EXTENT OF LSD BLOCKADE OF EFFECT OF IONTOPHORESED 5-HT FOR LSD GIVEN BY IONTOPHORESIS (Ip) AND INTRAVENOUS (i.v.) INJECTION

Extent of LSD induced blockade of 5-HT effect on spontaneous activity*.

*Response to** 5-HT and LSD*	*D*			*I*			*NR*			*Type of cell Extent of block. Total*
	2+	*1+*	*0*	*2+*	*1+*	*0*	*2+*	*1+*	*0*	
LSD applied by Ip										
5-HT ↑ LSD ↑	0	0	0	1	0	0	2	0	1	4
5-HT ↑ LSD ↓	9	3	1	1	0	0	0	0	0	14
5-HT ↑ LSD –	10	1	0	0	0	0	2	2	1	16
5-HT ↓ LSD ↑	0	0	0	0	0	1	0	0	3	4
5-HT ↓ LSD ↓	0	0	1	0	2	0	0	0	2	5
5-HT ↓ LSD –	0	0	0	0	1	0	2	1	1	5
Total LSD by Ip	19	4	2	2	3	1	6	3	8	48
*LSD given i.v.***,§*										
5-HT ↑ LSD ↑	1	0	0	0	0	0	0	0	0	1
5-HT ↑ LSD ↓	0	3	3	0	0	0	0	0	0	6
5-HT ↑ LSD –	2	4	0	0	0	0	0	0	0	6
5-HT ↓ LSD ↑	0	0	0	0	0	0	0	0	0	0
5-HT ↓ LSD ↓	0	0	1	0	0	3	0	0	0	4
5-HT ↓ LSD –	0	0	1	0	0	1	0	0	2	4
Total LSD i.p.	3	7	5	0	0	4	0	0	2	21

* Extent of blockade designated as 2 + = complete, 1 + = partial, 0 = none.
** Responses designated as ↑ = excitatory, ↓ = inhibitory, – = no response.
*** This group includes 5 units tested only by i.v. and 16 units also tested by Ip.
§ Dose of LSD given i.v. was 37 or 75 μg/kg (see text).

also tested by iontophoresis). The units are divided into D, I and NR cells as described above and responses of spontaneous neuronal activity to 5-HT and LSD are summarized along with the efficacy of LSD blockade of the effect of 5-HT applied by iontophoresis. Overall, 5-HT was excitatory for 35 of these units and inhibitory for 18 units. LSD was excitatory for 8 units, inhibitory for 22 and had no effect on 23 units. Of the 30 units for which LSD produced a response, the effects of 5-HT and LSD were similar in 13 instances (5 excitatory, 8 inhibitory) and opposite in 17.

There were 25 D cells in the group of 48 neurons to which LSD was applied by iontophoresis and LSD inhibited 14 of these D cells and had no effect on 11. LSD was administered by i.v. injection for 15 D cells, including 14 tested with iontophoresed LSD, and produced inhibition in 7, excitation in 1 and no effect in 7.

With regard to the I cells, LSD was applied by iontophoresis to 6 units and produced inhibition in 3, excitation in 2 and no effect in 1. LSD given i.v. inhibited 4 of 4 I cells tested.

For the NR cells, LSD applied by iontophoresis produced inhibition in 2,

excitation in 6 and no effect in 9 of the 17 units tested. LSD given i.v. had no effect on 2 NR cells.

Blockade of 5-HT excitation by iontophoresed LSD was tested on all of the 24 D cells excited by 5-HT. LSD produced complete blockade of 5-HT excitation in 19 (Table I, 2 + blockade) and partial blockade in 4 cells (Table I, 1 + blockade). Fig. 1 demonstrates blockade by iontophoresed LSD of 5-HT excitation on a D cell.

Of the 8 NR cells excited by 5-HT, iontophoresed LSD blocked this excitation in 6 (4 — 2+ block, 2 — 1+ block); LSD also blocked 5-HT excitation in 2 I cells that were excited by 5-HT.

Blockade of 5-HT inhibition by iontophoresed LSD was seen in only 6 of 14 neurons tested (Table I). Three of 4 I cells tested showed a weak blockade while one I cell and the one D cell inhibited by 5-HT showed no blockade. Only 3 of 9 NR cells demonstrated blockade of 5-HT inhibition by LSD, but the block was strong in two instances.

Intravenous injection of 37 μg/kg of LSD produced blockade of 5-HT excitation for 8 of 13 D cells tested with this dose while a larger dose of 75 μg/kg LSD blocked 5-HT excitation in an additional 2 of the 4 neurons not demonstrating blockade at 37 μg/kg. Intravenous LSD at a dose of 75 μg/kg did not block 5-HT inhibition in 8 units tested (4 I, 2 D, 2 NR cells).

The possibility that blockade of 5-HT action on neurons might be mediated through a local anesthetic effect caused by LSD[16] was investigated by monitoring action potential size and demonstrating response to a non-specific excitant, DL-homocysteic acid, during the period of LSD blockade. For the most part, LSD iontophoresis was done with a current of 40–80 nA, and local anesthetic effects were rarely seen. At stronger ejection currents, however, local anesthetic effects were sometimes noted. For intravenous LSD the doses of 37–75 μg/kg employed in most trials seldom produced local anesthetic effects; however, such effects were sometimes seen with higher doses.

Blockade by iontophoresed LSD of the putative excitatory 5-HT synapse on the D cell was tested on 18 D cells that were also excited by 5-HT. For these trials iontophoretic application of LSD was followed by iontophoresis of 5-HT and retesting of response to NPL stimulation. Simultaneous blockade of excitation by iontophoresed 5-HT and of synaptic excitation by NPL stimulation was seen in 16 units. Fig. 1 shows a D cell which demonstrates simultaneous blockade by LSD of synaptic excitation and also excitation by iontophoresed 5-HT. In all of these 18 D cells, the blockade by iontophoresed LSD of excitation by iontophoresed 5-HT was strong (2+). The synaptic blockade by LSD was measured by failure of a threshold stimulus to excite the cell (see above). Blockade was more than 70% complete (strong blockade) in 8 units, 30–70% complete (partial blockade) in another 8 units and absent in 2.

Blockade of synapse by intravenous LSD was tested in 5 units including one not tested with iontophoresed LSD. A dose of 37 μg/kg failed to block the synapse in 4 instances. A dose of 150 μg/kg produced simultaneous blockade of synaptic

excitation and excitation by iontophoresed 5-HT in 2 instances, including one neuron not tested with iontophoresed LSD.

Thus of the 19 D cells tested, simultaneous blockade by LSD of 5-HT excitation and of the synaptic excitation in response to NPL stimulation was seen in 17.

All of these 19 D cells remained excitable to suprathreshold stimuli during the period of LSD blockade and, in addition, responsiveness to DLH was maintained. In 2 instances a partial local anesthetic effect was suspected after 150 μg/kg of LSD given i.v. because of decrease in the amplitude of the extracellular action potential, but in the 18 units to which LSD was applied by iontophoresis, no evidence of local anesthetic effect was present.

Of the 9 I cells, trial of iontophoresed 5-HT and NPL stimulation was carried out in 6. Blockade by LSD of the inhibition produced by NPL stimulation was seen in 2 units. These were both units that showed excitation to 5-HT. In no instance was there simultaneous blockade of synaptic inhibition and inhibition by iontophoresed 5-HT.

On histological examination, the locations of a sample of 18 neurons (14 D cells, 4 NR cells) were determined. As in the previous work[9] all cells lay in the areas corresponding to the nucleus raphe pontis and caudal nucleus raphe medianus.

Most reports dealing with the effect of LSD on single neurons have indicated that the LSD produced inhibition in virtually all instances[5,15,19,20–23]. Foote *et al.*, however, found LSD applied by iontophoresis produced excitation in 22% of 45 non-raphe midbrain neurons tested[14]. In the present report, LSD inhibited 42% (22) and had no effect on 43% (23) while exciting 15% (8) of the 53 neurons in the pontine and lower midbrain raphe that were examined. The excitation was usually weak but was strong in 2 of the 8 units for which LSD produced excitation.

An inhibitory response to LSD has been reported to be a definitive characteristic of neurons of the dorsal raphe nucleus[1,2,15]. The responses to LSD of the raphe neurons studied here, which were primarily in the pons, were excitatory or no response in 59% of cases and inhibitory in only 41%. This suggests that the pontine raphe neurons constitute a different functional group than the neurons of the dorsal raphe nuclei.

The concept of heterogeneity of the pontine and dorsal raphe nuclei is supported by histochemical and degeneration studies. By histochemistry there are more 5-HT neurons in the dorsal raphe nucleus[13] and studies of raphe efferents demonstrated a primarily rostral projection for the dorsal raphe nucleus while the nucleus raphe pontis projects to the cerebellum[7]. These anatomic differences would suggest functional differences between pontine and dorsal raphe nuclei and consequently differences in response to LSD would not be surprising.

The D cell was originally postulated to receive an excitatory 5-HT synapse because of a high incidence of 5-HT excitation on neurons responding with synaptic excitation to NPL stimulation[9]. In work on the D cell, 100 D cells have been studied of which 97 were excited by 5-HT[9,10].

In addition to these physiologic and pharmacologic data, an anatomic basis for an NPL-to-raphe serotonergic pathway can be inferred from histochemical and

degeneration studies. Since the NPL lies within the B-3 group of 5-HT neurons described by Dahlström and Fuxe[13], 5-HT neurons are present at the point of stimulation in these experiments and could be the origin of the proposed pathway. Studies reported by Bloom *et al.* demonstrated that lesions of the NPL would produce degeneration of nerve endings in the pontine raphe suggesting anatomic projection of NPL neurons to the raphe[4]. Bloom *et al.* also reported that nerve endings in the pontine raphe would take up [^{3}H]5-HT from intracisternal injection[4]. In summary, there appears to be 5-HT neurons in the NPL, anatomic connection from NPL to raphe and terminals at the pontine raphe that are capable of taking up 5-HT. With this background, the demonstration of excitatory synaptic input on D cells from an area containing 5-HT neurons and an excitatory response to iontophoresed 5-HT for 97% of these D cells provides a strong suggestion that the D cell has an excitatory 5-HT synapse impinging on it.

In 17 of 19 D cells, LSD was shown to block simultaneously synaptic excitation and excitation by iontophoresed 5-HT. These data provide a strong suggestion that LSD exerts a specific blockade on this excitatory central 5-HT synapse.

In studies employing iontophoresis, LSD has been found to mimic and not block inhibitory effects of 5-HT in cortex[19,22], thalamus[20], lateral geniculate nucleus [21,25] and raphe nuclei[2,15]. These findings, in conjunction with studies showing that LSD will decrease 5-HT turnover[8], have led to the suggestion that LSD acts by stimulation of inhibitory central 5-HT receptors.

The data on I cells reported here are in line with the studies noted above. Six of 9 I cells were inhibited by LSD, one did not respond and two were excited. Blockade of the postulated inhibitory 5-HT synapse was not seen.

The action of LSD on excitatory 5-HT synapses, however, appears to be one of blockade and not stimulation of the postsynaptic receptor. Of the 24 D cells excited by 5-HT, none were excited by iontophoresed LSD but one was excited by i.v. LSD. Similarly, Boakes *et al.* in their study of reticular formation neurons did not find that LSD would mimic 5-HT excitation[5]. Of 19 D cells tested for synaptic blockade, LSD simultaneously blocked synaptic and 5-HT excitation in 17. These data suggest that with regard to the D cell, LSD acts by blocking the excitatory 5-HT synapse and not by stimulation of the postsynaptic receptor.

The studies of the effect of LSD on central 5-HT systems have suggested several possible mechanisms of action. Boakes *et al.*[5] and Bradley and Briggs[6] have postulated that the primary effect of LSD is blockade of excitatory 5-HT synapses. Andén *et al.*[3] and Aghajanian and Haigler[1,2] have suggested that the major effect of LSD is to stimulate inhibitory 5-HT receptors. The work here is compatible with both concepts. This suggests that LSD might have a dual effect on central serotonergic systems acting to block excitatory 5-HT synapses and stimulate inhibitory 5-HT receptors.

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