Life Sci. 11, Part I, pp.615-622 (1972).

Life Sciences Vol. 11, Part I, pp. 615-622, 1972. Pergamon Press
Printed in Great Britain

LYSERGIC ACID DIETHYLAMIDE AND SEROTONIN: DIRECT ACTIONS ON SEROTONIN-CONTAINING NEURONS IN RAT BRAIN

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(Received 17 March 1972; in final form 10 May 1972)

Summary

Lysergic acid diethylamide (LSD) and serotonin (5HT) were applied directly by microiontophoresis to 5HT-containing neurons in the midbrain raphe nuclei. The firing of these neurons was markedly inhibited by both LSD and 5HT. The effects of LSD given systemically and microiontophoretically were similar. This suggests that the inhibition of raphe neurons which occurs after the systemic administration of LSD could be due to a direct action of the drug.

SMALL doses of d-lysergic acid diethylamide (LSD) given by the systemic route inhibit the firing of neurons in the midbrain raphe nuclei (1, 2). There is histochemical evidence that the neurons within the raphe nuclei contain serotonin (5-hydroxytryptamine; 5HT) and possibly other indoleamines (3, 4, 5). The finding that LSD can alter the rate of firing of 5HT-containing (i.e., raphe) neurons is of interest for the hypothesis that LSD might produce its behavioral effects by interacting with 5HT in the brain (6, 7). In the above studies, since the systemic route of administration was used, the action of LSD on raphe neurons may be direct or indirect. To determine if LSD can directly depress the firing of raphe neurons the drug was applied locally via multibarreled micropipettes. In addition, the effect of 5HT applied directly to raphe neurons was investigated because systemic treatments which increase brain 5HT concentration and the intensity of histofluorescence of raphe neurons (3, 4) also depress raphe unit firing (8, 9).

Methods

A total of 46 male albino rats (Charles River, 225-275 g) was used. The animals were either anesthetized with chloral hydrate (400 mg/kg) or were maintained without anesthesia in a "pretrigeminal" preparation (10). Rats were placed in a stereotaxic instrument and burr holes were drilled in the midline over the caudal midbrain; electrodes were positioned as previously described (1, 2). Extracellular recordings, iontophoretic ejection of drugs, and maintenance of current balance were by methods similar to those described by Salmoiraghi and Weight (11). The tips of 5-barreled micropipettes were broken back to a diameter of 3 to 4 μm and solutions were introduced into the various barrels by direct injection; tips became filled by capillary action due to the presence of fiberglass (12). Two of the 5 barrels, those for recording and current balance were filled with 2M NaCl saturated with fast green dye for marking purposes (15). Concentrations of drugs in barrels were as follows: D-LSD bitartrate, 5×10^{-4}M in 0.05 M NaCl (pH 3.5); 5HT creatinine sulfate, 5×10^{-2}M (pH 3.5); 2-bromolysergic acid diethylamide bitartrate (BOL), 5×10^{-4}M or 5×10^{-3}M in 0.05 M NaCl (pH 3.5); sodium L-glutamate, 0.5 M (pH 8.5); l-norepinephrine bitartrate, 5×10^{-2}M (pH 3.5); and NaCl 0.15 M (pH 3.5). Some leakage effects have been found in previous iontophoretic studies with LSD (13). Therefore, the drug was used in a dilute solution to avoid the need for excessive holding currents. NaCl was added to facilitate passage of ejecting current, as suggested by Curtis and Crawford (14). Holding currents were used when necessary and current balance (i.e., sum of all currents) was kept to within $\pm$ 5 nanoamperes. Impedences, measured at 1,000 hertz, were usually 15 to 20 megohms in the recording barrels and slightly higher in the drug barrels. Signals were passed through a high input-impedence amplifier and displayed on an oscilloscope. Recordings consisted of consecutive 10 second samples of the analog output of an electronic counter triggered by individual neuronal spikes (2).

Upon completion of each experiment, fast green was ejected through the recording or balance barrel (15). For histological determination of recording sites animals were perfused with fixative (5% glutaraldehyde in 0.9% saline) and frozen sections of midbrain were cut, mounted, and stained with neutral red. In order to confirm the reported location of 5HT neurons (3) histochemical fluorescence studies were carried out in control animals by a modification (4) of the method of Falck et al. (16).

Results

Of 85 neurons studied, 63 were located in the dorsal and 4 in the median raphe nucleus of the midbrain. We found, in agreement with Dahlstrom and Fuxe (3), that these areas had a high density of yellow-fluorescent neurons. The raphe neurons, both in the anesthetized and unanesthetized animals, had the typical slow rate (0.5 to 2.0 spikes/sec.) and regular rhythm previously described for raphe neurons in chloral hydrate anesthetized rats (1, 2). Without exception, the raphe neurons were inhibited by the microiontophoretic application of LSD or 5HT (Fig. 1A). In anesthetized animals, the mean ejection currents required for complete inhibition of firing by LSD and 5HT were respectively 20 (range - 4 to 100; n = 39) and 6 (range - 2 to 16; n = 29) nanoamperes. When submaximal amounts of LSD and 5HT were ejected simultaneously their combined inhibitory effects were additive. There was no indication that LSD in any way blocked the action of serotonin since effects were always inhibitory regardless of whether the two compounds were ejected alternately or simultaneously. No effects were seen with the ejection of Na^+ or Cl^- ion alone. BOL, an analogue of LSD with little behavioral potency, had no effect on the firing of raphe neurons despite ejection currents 10 times greater than used for LSD. In addition, BOL, which is a serotonin antagonist in the periphery (17), did not block the inhibitory effect of serotonin on raphe cells. The actions of l-norepinephrine were variable; of 28 raphe cells studied with this amine, 12 were excited, 10 were inhibited;

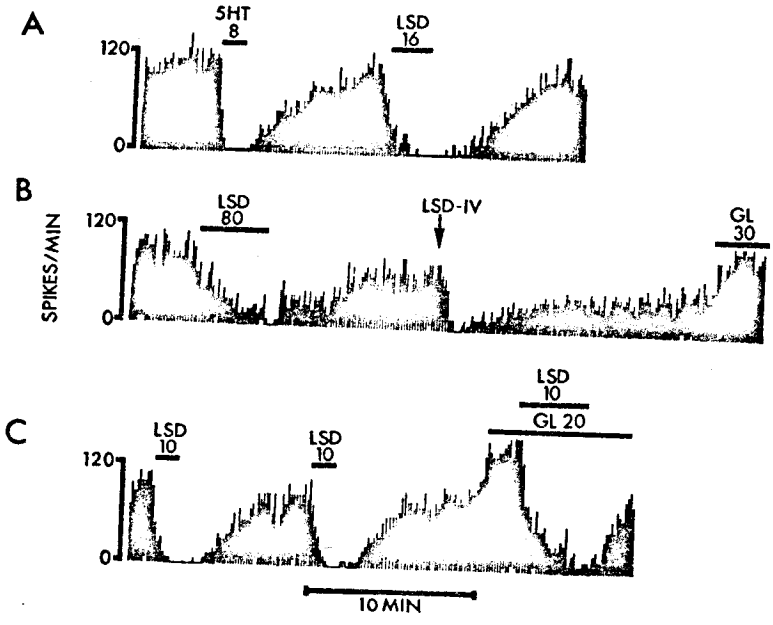

Fig. 1

Effects of LSD, 5HT, and glutamate (GL) on midbrain raphe neurons. (A) 5HT and LSD, given sequentially, are both seen to have an inhibitory effect on rate of firing. The number and line below label for each substance indicate respectively microiontophoretic ejection current (in nanoamperes) and duration of ejection.
(B) LSD given microiontophoretically and intravenously (I.V., 20 µg/kg) are seen to have a similar inhibitory action upon the same raphe unit. Glutamate, given at a time when recovery from the I.V. LSD is incomplete, restores firing rate to original level.
(C) LSD ejected twice in succession has a reproducible inhibitory action on a raphe cell. The ejection of glutamate results in approximately a doubling of rate. LSD is able to overcome the glutamate excitation, but the ultimate degree of inhibition is not as great as it was prior to glutamate ejection.

the remainder did not respond. In unanesthetized (pretrigeminal) preparations, responses to iontophoretically applied LSD and 5HT were as described above except that somewhat higher ejection current was usually required for complete inhibition than in the anesthetized animals. Most of the 19 midbrain neurons tested outside the raphe nuclei (e.g., reticular formation, pontine nuclei, or ventrolateral central gray) were unaffected by LSD or 5HT at the same ejection currents used for raphe cells. However, four of these

cells were excited and two slightly inhibited by 5HT.

In some experiments, the response of raphe neurons to microiontophoretically ejected LSD was compared to that of small intravenous doses of the drug. The overall effect of LSD given by these different routes was found to be the same (Fig. 1B). There was no decrease in spike amplitude when slowing was induced by either intravenous or iontophoretic application of LSD. Because of reported interactions between l-glutamate and LSD in certain brain stem neurons (18), the possibility of such interactions was examined in raphe neurons. The excitatory effect of l-glutamate could be reversed partly by LSD (Fig. 1C). However, an increased ejection of l-glutamate was capable of overcoming LSD inhibition. LSD did not block glutamate excitation in the non-raphe cells tested. Interestingly, the excitation of raphe cells by l-glutamate could also be partly overcome by the intravenous injection of LSD (20-40 μg/kg).

Discussion

The above results show that midbrain raphe neurons are markedly inhibited by the direct, microiontophoretic application of LSD at ejecting currents which do not inhibit other midbrain neurons. These results suggest that the inhibitory action on raphe neurons of LSD given by the systemic route may be due to a direct effect on these cells. A similarity between the effects of the local and systemic administration of LSD on raphe neurons is further indicated by the fact that in both cases l-glutamate excitation can be partly reversed. In other midbrain neurons tested, LSD did not block l-glutamate excitation. Furthermore, after parenteral or iontophoretic administration of LSD, there was no decrease in the spike amplitude of raphe cells. Therefore, it seems unlikely that the effect of LSD on raphe cells is due to a nonspecific or local anesthetic action (19, 20). Biochemical studies show that LSD given systemically decreases the turnover of 5HT in brain (21, 22, 23). It has been suggested that the concomitant depression in the firing of

raphe neurons caused by LSD may account for this decrease in turnover (1, 2). The possibility that this is a direct effect of the drug is consistent with the finding that the implantation of minute crystals of LSD in the raphe nuclei can result in a similar decrease in 5HT turnover (24). Nevertheless, it is still possible that LSD given systemically may also influence raphe neurons indirectly, possibly through a neuronal feedback mechanism (25, 26).

The finding that midbrain raphe neurons are inhibited by 5HT given iontophoretically is consistent with previous results showing that agents which increase brain 5HT content and the fluorescence of raphe cells, either by preventing catabolism (e.g., monoamine oxidase inhibitors) or by accelerating synthesis (e.g., L-tryptophan), depress the firing of raphe neurons (8, 9). Our results seem to differ from those of Couch, who reported that in pontine raphe neurons responses to 5HT were often excitatory (27). However, in the latter study many of the pontine raphe cells had high rates of discharge and this is not typical of the raphe units we have studied in the midbrain (1). Moreover, fluorescent neurons are not as highly concentrated in the pontine raphe as they are in the dorsal raphe of the midbrain (3). It would thus appear that these discrepant results can be explained by a difference in the population of the cells examined. In any case, the possible physiological significance of inhibitory responses to serotonin among midbrain raphe cells is uncertain. Since 5HT may be the transmitter substance of these neurons, it is conceivable that 5HT may be involved in an inhibitory recurrent collateral input upon the midbrain raphe. Uptake of tritiated 5HT has been observed in nerve terminals in the area of the midbrain (28) and pontine (29) raphe nuclei by electron microscope autoradiography.

The similarity between the effects of LSD and 5HT on raphe neurons is of interest in relation to hypotheses about the mechanism of action of this drug in brain. There are recent studies, using microiontophoretic techniques, showing that LSD can block the excitatory effects of 5HT on cortical (30) and

brain stem neurons (18) in unanesthetized animals. The latter observations seem consistent with the hypothesis that LSD is a 5HT antagonist in brain. However, in view of the similarity of the effects of LSD and 5HT on midbrain raphe cells, the general question of whether LSD blocks or mimics 5HT in the central nervous system would seem to require further exploration.

Acknowledgement - We thank the FDA-PHS Psychotomimetics Agents Advisory Committee for providing the LSD used in these studies. Supported in part by NIMH Grants (MH 17871 and MH 14459) and the State of Connecticut.

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