Life Sci. 5, 259-266 (1966). LSD 1399

Life Sciences Vol. 5, pp. 259-266, 1966. Pergamon Press Ltd.
Printed in Great Britain.

THE EFFECTS OF LSD-25 ON THE AMPLITUDES OF EVOKED POTENTIALS IN THE HIPPOCAMPUS OF THE CAT

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(Received 6 December 1965)

Administration of reserpine to cats causes seizure activity in hippocampus (1,2) and a facilitation of the amygdalo-hippocampal evoked potential (3). These actions of reserpine on the hippocampus seem related to the depletion of brain serotonin (5HT) induced by the drug (4,5). 5HT and tryptamine are inhibitory to hippocampal neurones (6,7), and depletion of an inhibitory "neurohormone" would be expected to increase the excitability of the hippocampus. If 5HT is an inhibitory "neurohormone", then lysergic acid diethylamide (LSD), which antagonizes the actions of 5HT at most peripheral synapses (8), should have effects on the hippocampus which are similar or identical to those of reserpine. Indeed, seizure activity has been found in cat hippocampus following administration of small doses of LSD (1,2). The following experiments were performed to determine whether the effects of LSD on evoked potentials in the hippocampus were similar to those of reserpine.

Methods

Twelve adult cats weighing 2.5 - 3.5 kg were used. The animals were anesthetised with ether and placed in a stereotaxic instrument. The mesencephalic reticular formation at A 1.5 was then destroyed by a series of electrolytic fulgurations, and the ether was discontinued. The resulting unconscious modified cerveau isole' cat could be kept in good condition for about a week, with rectal temperatures maintained at 35°C - 36°C, and routine antibiotic and fluid therapy. Thus, when desirable, the effects of repeated drug admin-

istrations could be compared in one animal, free from possible anesthetic interference. Stimulating and recording electrode pairs were made from insulated stainless steel wire. The tip dimensions were 0.25mm x 0.50mm; the tip to tip distance was about 1.0mm. The electrode pairs were placed bilaterally in ventral hippocampus and in the right lateral amygdalar nucleus and the right septal area. Electrode positioning was guided by stereotaxic coordinates (9) and by observation of evoked potential characteristics during placement. After final positioning the electrodes were fixed to the skull with dental acrylic. All electrode positions were confirmed histologically after the experiment.

Spontaneous electrical activity and evoked responses were routinely recorded from right ventral hippocampus. Stimulation of amygdala, septum and contralateral hippocampus produced evoked potentials in the hippocampus, which were derived from three independent pathways and which terminated in characteristic intra-hippocampal synaptic fields (10). By analyzing the drug effects in these 3 pathways the existence of selective drug actions could be determined.

Previous experience with this preparation has shown that hippocampal excitability varies markedly and unpredictably for some time after electrode implantation. Most of the fluctuation in excitability occurs within 8-10 hours of electrode positioning, although full stabilization of excitability is not seen until 5-7 days after implantation. For this reason recordings were delayed for at least 18 hours after electrode positioning and were seldom taken for more than 8 hours continuously. In general, control records were taken for 2 to 3 hours, the drug was injected, and the recordings continued from 4 to 6 hours thereafter. Stimulus intensities used ranged from threshold to intensities which evoked responses of 1/2 maximal amplitude. Stronger stimuli were avoided due to the risk of triggering hippocampal seizures.

An average response computer (Nuclear Data ND-800) was used in order to simplify data analysis. 125 evoked responses were averaged for each data

point. In control recordings taken over an 8 hour period the maximum deviations from the mean potential amplitude were ±5%. Therefore, in the experiments in which drugs were given, changes in mean evoked response amplitude of less than ±10% of the mean control amplitudes were not considered significant. Changes of 10-20% were considered "possibly significant" and changes of more than 20% were considered "significant" ($p<0.01$).

The amplifying, stimulating, photographic, and ink recording systems were conventional. LSD and pentobarbital were obtained commercially. The drugs were dissolved in 0.9% NaCl and injected intravenously.

Results

Characteristics of the Evoked Potentials. The characteristics of the potentials evoked in the hippocampus by stimulation of amygdala, septum, and contralateral hippocampus have been described by many authors (10). Typical potentials obtained in the present study are illustrated in Figure 1. The septo-hippocampal evoked potential (SHEP) and the trans-hippocampal evoked potential (THEP) are very similar in latency and wave-shape. The amygdalo-hippocampal evoked potential (AHEP) usually differs from SHEP and THEP in latency, duration, and waveshape, although the potentials shown in Figure 1 differ less markedly than is usual. The pathway for the AHEP is poly-synaptic, unlike the mono- or oligo-synaptic SHEP and THEP. The intra-hippocampal terminations producing AHEP are those of the perforant pathway and differ considerably from the synaptic sites producing SHEP and THEP (10).

Saline Effects. Administration of 1-2ml of physiological saline to two cats in this series did not change any of the evoked potential amplitudes.

Pentobarbital Effects. Pentobarbital, in doses of 20mg/kg, was injected into 3 cats on 5 separate days. This was done so that the activity pattern, if any, of LSD-25 could be compared to the patterns induced by a drug of known pharmacological effectiveness. Pentobarbital reduced the amplitude of the AHEP by about 35%. Amplitudes of the SHEP and the THEP were also reduced, but only by 15% and 10% respectively. There was, however, considerable variability in the

degree of response to pentobarbital. Smaller doses of pentobarbital (10mg/kg) could produce a slight facilitation (15%) of AHEP, with minimal effects on SHEP and THEP. Facilitation of hippocampal electrical activity at low doses of pentobarbital and inhibition at higher doses was also reported by Bradley and Nicholson (11).

LSD-25. Doses of 50γ/kg or LSD-25 were without effect on the potentials

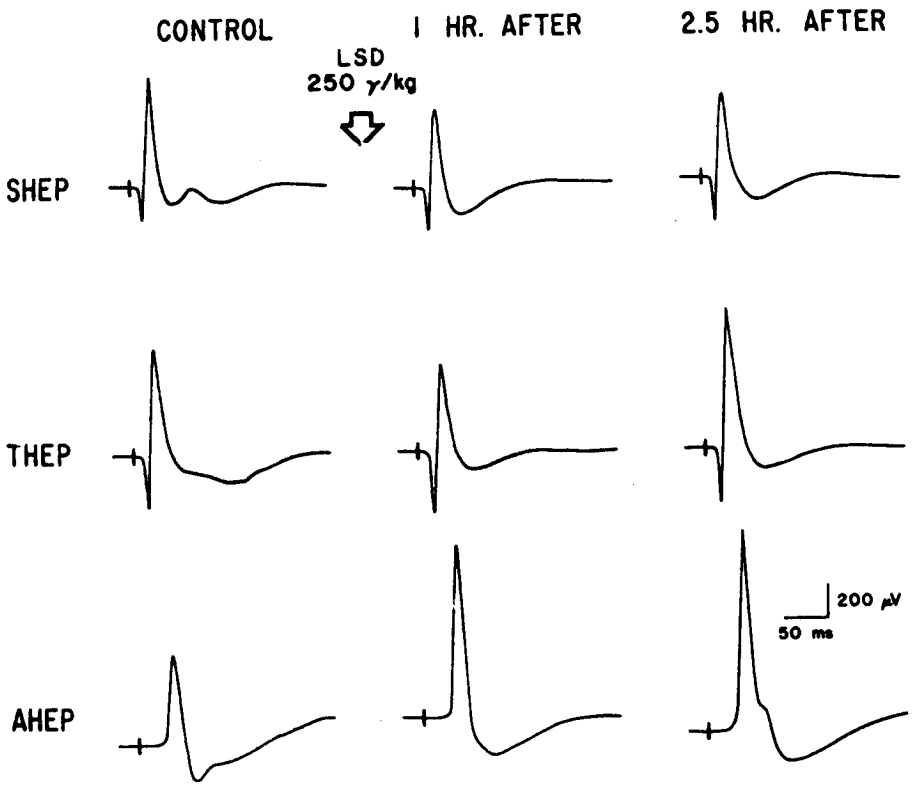

FIG. 1

Hippocampal evoked potentials before and at two times after administration of LSD. SHEP=septo-hippocampal evoked potential; THEP=trans-hippocampal evoked potential; AHEP=amygdalo-hippocampal evoked potential. The individual records above are tracings from the computer write-out.

studied. LSD in doses of 100-250γ/kg induced a marked facilitation (>40%) of the AHEP in 5 animals. These doses of LSD either had no effect upon SHEP and THEP responses or caused a slight (10-15%) inhibition (Fig. 1). The AHEP facilitation was seen only following the first injection of LSD-25. Further injections of LSD-25 were without effect on the evoked potentials unless toxic drug levels were reached. Doses of LSD-25 larger than 250γ/kg caused a rapid and complete inhibition of all hippocampal electrical activity, spontaneous and evoked. Although most animals appeared in good condition for at least two hours after injection of these large doses of LSD-25, the animals died three to seven hours after injection. In two instances some recovery of the SHEP and THEP was seen two to three hours after injection of 400γ/kg of LSD-25, although the animals died 1-3 hours thereafter.

The responses to LSD-25 were variable. Five animals showed facilitation of the AHEP after 100-250γ/kg of LSD-25. However, two cases of inhibition and one of no change were observed after injection of 250γ/kg of LSD-25. In the latter 3 cases, however, relatively small doses of LSD-25 (100-250γ/kg) given the next day were sufficient to induce complete electrical silence in hippocampus and, after 4 hours, death. One cat given 100γ/kg in divided doses, 2 hours apart, also went into electrical silence and died 3.5 hours after the second LSD-25 injection.

Discussion

The present results indicate that LSD has a diphasic action on the AHEP. LSD in doses of 100-250γ/kg facilitated the AHEP; doses >250γ/kg inhibited the AHEP. Previous studies on the effects of LSD upon spontaneous electrical activity in the hippocampus have shown a similar diphasic dose-response characteristic (1,2). Considerable variability in the response of the AHEP to LSD was seen, but was not unexpected. The preparations certainly differed considerably in their "sensitivity" towards LSD, for the lethal dose of the drug ranged from 100-800γ/kg. Given a diphasic dose-response characteristic, even small variations in "sensitivity" can cause marked variations in drug

effects in the region of transition from excitation to inhibition. Furthermore, previous studies of the effects of drugs on the AHEP in chronic preparations has shown that, although drug effects are stable and reproducible in any one animal, there is considerable variation in the drug effects among the animals (3). There was no apparent reason for the intra-animal variability, in this study or the previous one (3), either in terms of behavioural responses to the drug or in terms of methodological variations such as electrode location.

The LSD-induced increase in hippocampal electrical activity seen in previous studies (1,2) has been attributed to a direct excitant effect of the drug on hippocampal neurones. However, it is also possible that the hippocampal excitation caused by LSD is due to inhibition of inhibitory pathways within or afferent to the hippocampus. If so, and noting the diphasic dose-response characteristic of LSD, the following hypothesis can be formed: LSD is purely inhibitory to all or most neurones in hippocampus, but certain inhibitory cells or endings are the most sensitive to the drug. Therefore, at low doses of LSD some excitatory phenomena may be seen, but higher doses will be purely inhibitory. There is evidence for this hypothesis.

The effects of LSD on the THEP and the SHEP are purely inhibitory (Fig. 1). This confirms and extends previous work showing that LSD blocks septo-hippocampal transmission (12). Therefore, the drug can not have a direct, excitatory action on hippocampal neurones in general. Also, the "inhibition of inhibition", if present, cannot be a general intra-hippocampal phenomenon, since, again, no excitation phase has been seen in SHEP or THEP. As mentioned above, LSD in sufficient quantities, inhibited hippocampal electrogenesis (1, 2, 12). The doses of LSD used in these experiments (1,2,12) were not reported to be toxic, so the observed inhibition probably represents a normal pharmacological effect of LSD. In the present experiments, LSD inhibited hippocampal evoked potentials at doses >250γ/kg. Indeed, complete hippocampal electrical silence could be produced by these large doses. However, these

doses were lethal, or nearly so, and the inhibition we found could be a toxic effect of the drug. However, although the electrical silence developed within 15 minutes of drug injection, the condition of the animal did not begin to deteriorate for several hours. Thus, the inhibition and toxicity may not be related. Furthermore, experiments on the effects of amphetamine in reserpinized cats have shown that complete hippocampal electrical silence over a period of hours can be induced by drug doses which are neither lethal nor toxic (3). Consequently, we feel that the inhibition of hippocampal electrical activity seen in the present study was a pharmacological action of LSD and not a toxic effect. There is also some indirect evidence for an inhibitory action of LSD in hippocampus. The present study and previous work (11) have indicated that pentobarbital, a general neuronal inhibitor, had a diphasic action in the hippocampus which resembles that of LSD.

The data noted above supports the hypothesis that LSD is an inhibitory agent in the hippocampus. The facilitation of evoked activity seen following low doses of LSD was seen only in AHEP. Therefore, it is probable that the structures most sensitive to LSD inhibition in the hippocampus are inhibitory neurones in the polysynaptic amygdalo-hippocampal pathway, possibly in the entorhinal area (1).

The original hypothesis which stimulated this work was that LSD and reserpine should have similar actions on the hippocampus since both could be taken as functional antagonists of 5HT. However, from the preceding discussion, it seems probable that LSD is an inhibitor of hippocampal neurones, as is 5HT (6,7). Thus, LSD may be a 5HT mimic in this system, as it is at some peripheral synapses (8). Therefore, the original hypothesis is untenable and the present experiments do not help explain the mode of action of reserpine on the hippocampus.

Summary

LSD in doses of 100-250γ/kg facilitated the amygdalo-hippocampal evoked potential. Doses equal to or larger than 250γ/kg inhibited the amygdalo-hippo-

campal response, the trans-hippocampal evoked response and the septo-hippocampal evoked potential. The data is interpreted to indicate that LSD is a purely inhibitory compound in the hippocampus.

References

1. W. R. Adey, F. R. Bell, and B. J. Dennis, Neurol. 12, 591 (1962).
2. K. F. Killam, and E. K. Killam, J. Pharmacol. Exper. Therap. 116, 35 (1960).
3. E. Costa, C. Morpurgo, and A. M. Revzin, Recent Advances in Biological Psychiatry 3, 122 (1960).
4. A. M. Revzin, S. Spector, and E. Costa, Int. J. Neuropharmacol. 1, 85 (1962).
5. A. M. Revzin, R. P. Maickel, and E. Costa, Life Sciences 1, 699 (1962).
6. L. deBaran, G. Gogolak, V. G. Longo, and Ch. Stumpf, J. Pharmacol. Exper. Therap. 139, 337 (1963).
7. T. J. Biscoe, and D. W. Straughan, J. Pharm. Pharmacol. 17, 60 (1965).
8. L. Gyermek, Pharmacol. Rev. 13, 399 (1961).
9. H. H. Jasper, and C. Ajmone-Marsan, A Stereotaxic Atlas of the Diencephalon of the Cat. National Research Council of Canada, Ottawa (1954)
10. J. D. Green, Physiol. Rev. 44, 561 (1964).
11. P. B. Bradley, and A. N. Nicholson, Electroenceph. Clin. Neurophysiol. 14, 824 (1962).
12. C. Stumpf, H. Petsche, and G. Gogolak, Electroenceph. Clin. Neurophysiol. 14, 212 (1962).