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PRELIMINARY NOTES

SELECTIVE BLOCKING ACTION OF LSD ON INHIBITORY DOPAMINE RECEPTORS

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Summary—Dopamine-induced postsynaptic responses of both excitory and inhibitory types were recorded from identified neurones of *Aplysia* ganglion. A 30 sec exposure to 0·1 μM D-lysergic acid diethylamide (LSD) significantly depressed the inhibitory responses to 1 mM dopamine, while a 60 sec exposure to 10 μM LSD did not alter the excitatory responses to 0·1 mM dopamine. Dose–response curves obtained from cells with inhibitory receptors indicate that the mode of LSD interaction was non-competitive.

D-Lysergic acid diethylamide (LSD-25) has been demonstrated to antagonize (ROBERTS and STRAUGHAN, 1967) or mimic (ANDEN, CORRODI, FUXE and HÖKFELT, 1968) serotonergic transmitter in the central nervous system (CNS), while some investigators consider that the central effect of LSD has nothing to do with serotonergic transmission (COSTA, GESSA, HIRSCH, KUNTZMAN and BRODIE, 1962). Despite considerable work, the primary action and the acting site of LSD in the CNS remains controversial (AGHAJANIAN, 1972). Recently, HUNGEN, ROBERTS and HILL (1974) reported that D-LSD stimulates the adenyl cyclase activity in corpus striatum of rats but it blocks the dopamine-induced activation of this enzyme. A behavioural study of the rat (PIERI and HAEFELY, 1974) indicated that D-LSD may act as an agonist on the dopamine receptors in the striatum of the rat. These observations suggested a certain interaction of LSD with the central dopamine receptors. The abdominal and pleural ganglia of *Aplysia* include identified neurones sensitive to acetylcholine, dopamine, noradrenaline, serotonin and γ-aminobutyric acid (GABA). They are a useful model for the analysis of drug actions on various receptors. Certain evidence indicates that acetylcholine and dopamine are possible natural transmitters in this system (GERSCHENFELD, 1973). We have examined the effects of LSD-25 on various kinds of receptors and have found that inhibitory dopamine receptors are only sensitive to LSD-25 (hereinafter simply LSD).

METHODS

The abdominal or pleural ganglion of *Aplysia californica* was dissected and fixed in a perfusing chamber. The connective tissue covering the dorsal surface of the ganglion was carefully removed to expose the identified neurones directly to the perfusing media. Cells were normally perfused with artificial *Aplysia* Ringer (SATO, AUSTIN, YAI and MARUHASHI, 1968); the size of the perfusion pool was 0·2 ml, flow rate 5 ml per min, pH 7·4 and temperature 10°C. Two microelectrodes were inserted into single identified neurones to record both the membrane potential and conductance. All chemicals were dissolved in *Aplysia* Ringer and applied directly to the cell under study through a constant flow of microperfusion. The responses of the cell to various chemicals were evaluated by the changes in membrane potential (ΔE) and conductance (ΔG). Details of this method have been published elsewhere (SATO *et al.*, 1968). The blocking potency of LSD was compared among different types of receptors in different cells (23 cells for dopamine-, 6 cells each for serotonin-, acetylcholine-, noradrenaline- and GABA-receptors). The excitatory (depolarizing) type of responses to dopamine were obtained from cells in

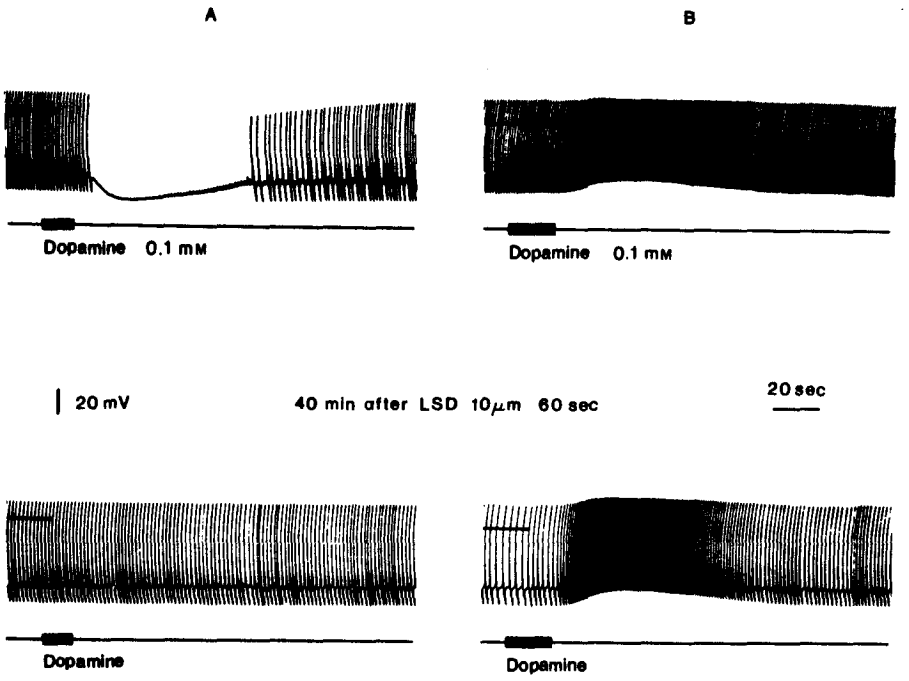

Fig. 1. A: Dopamine-induced hyperpolarizing response recorded from a cell in the R_B group of the abdominal ganglion. Note the disappearance of the spontaneous spike firing. B: Dopamine-induced depolarizing response recorded from a cell in the anterior group of the pleural ganglion. In both A and B, bottom traces were recorded 40 min after a 60 sec application of 10 μM LSD. Membrane resting potentials are shown by the horizontal bar on the top left of each trace.

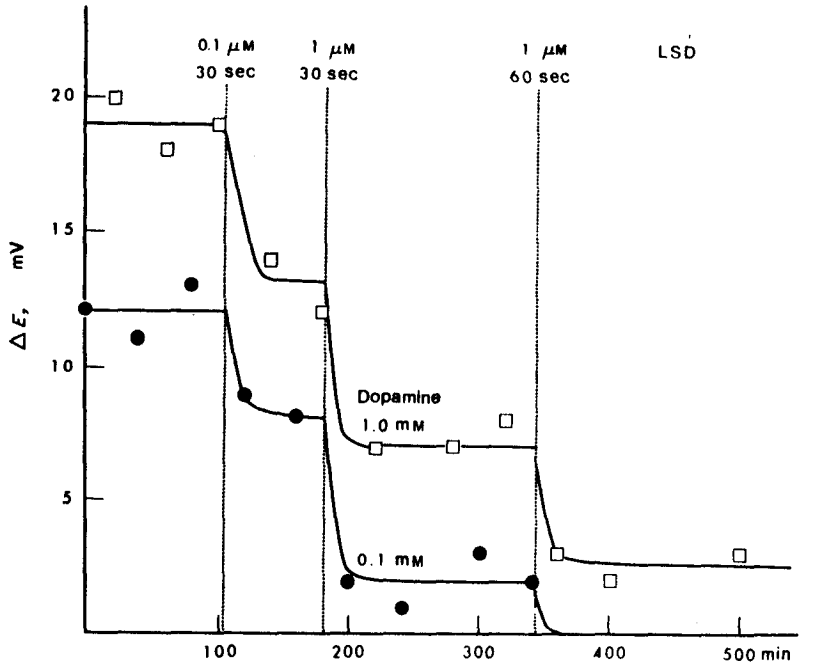

Fig. 2. Time course of the dopamine-induced responses after exposure to LSD. The responses were measured from the amplitude of hyperpolarization (ΔE) in mV. LSD concentrations and time of exposure to LSD are indicated on the top. The empty squares and filled circles are responses of the same membrane to dopamine of 1·0 and 0·1 mM, respectively.

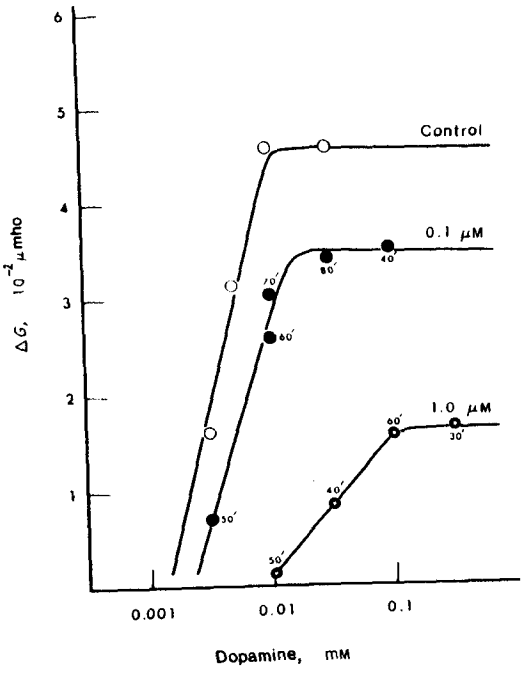

Fig. 3. Effects of LSD on dopamine-induced responses. Dose–response curves were obtained from a cell which was hyperpolarized by dopamine. The response was evaluated by the peak increase in membrane conductance (ΔG) produced by a given concentration of dopamine. The time of exposure to LSD was 60 sec and the LSD concentrations are shown on each curve. The time of measurement after each exposure to LSD is indicated at each point in min.

the anterior portion of the pleural ganglion, whereas the inhibitory (hyperpolarizing) type of responses to dopamine were recorded from neurones in the R_B group of the abdominal ganglion. Responses to other transmitters were from cells either in the R_B or R_C group of the abdominal ganglion.

RESULTS

Figure 1 illustrates a marked difference in susceptibility to LSD between two types of dopamine receptors. A 60 sec application of 10 μM LSD completely blocked the inhibitory type but did not affect the excitatory type of dopamine-induced responses. A higher sensitivity of the inhibitory response to LSD and its dose- and time-dependence are shown in Figure 2. A significant depression of the inhibitory response was seen after a 30 sec application of 0·1 μm LSD. A 60 sec application of 1 μm LSD completely blocked the hyperpolarizing responses to 0·1 mM dopamine. The application of less than 10 μM LSD did not alter the resting membrane potential or conductance. It should be noted that the onset of depression took place rather gradually over a period of 20–30 min before reaching the maximum depression plateau. The blockade lasted more than 2 hr and during this time the preparation was continuously washed with normal Ringer. This depression was not irreversible and the recovery of the response took place gradually over a period of 5–8 hr (not illustrated).

In order to analyze the mode of LSD inhibition, a series of dose–response curves were obtained from the single nerve cell using different concentrations of LSD and dopamine (see Fig. 3). In this experiment, the dopamine-induced responses were evaluated by the peak increase in membrane conductance (ΔG). The decrease in slope associated with the fall in the maximum plateau of the curve suggested that the mode of LSD inhibition was of the non-competitive type.

The blocking effects of LSD on other kinds of receptor activities were not significant. The serotonin-induced responses of the excitatory type were not appreciably depressed even after 2 min application of 100 μM LSD. The serotonin-induced responses of the

inhibitory type were not studied. Neither the excitatory nor the inhibitory type of responses elicited by acetylcholine, noradrenaline and GABA was affected by 5 min application of 100 μM LSD.

DISCUSSION

From the above results, it was concluded that LSD selectively blocks the inhibitory type of dopamine receptors in the ganglion cells of *Aplysia*. The mode of LSD interaction with receptors is non-competitive. Because of the structural resemblance between LSD and ergometrine, similar selective effects may be expected. WALKER, WOODRUFF, GLAIZNER, SEDDEN and KERKUT (1968) demonstrated with snail neurones that ergometrine antagonizes the inhibitory action of dopamine. BERRY and COTTRELL (1973) observed in *Planorbis* neurones that ergometrine blocked both the inhibitory and the excitatory types of dopamine-responses; the blocking potency being 20 times higher for the former type. ASCHER (1972) observed in *Aplysia* ganglion cells that 10 μM methylergometrine blocked the dopamine-induced inhibitory type of response but not the excitatory type. It should be noted that our results obtained with LSD closely resembled the selective effect of methylergometrine observed by ASCHER (1972), although the blocking potency of LSD appeared to be at least 100 times higher.

GERSCHENFELD and PAUPARDIN-TRITSCH (1974) analyzed the effects of LSD on the serotonin receptors in molluscan ganglion cells and found that the excitatory responses of the fast type or type A were readily blocked by LSD but those of the slow type or type A′ were not. In fact, the serotonin-induced responses observed in this present study were of the slow type, thus confirming the observation of GERSCHENFELD and PAUPARDIN-TRITSCH (1974).

The extremely high sensitivity, the long lasting effect and the gradual onset of the blockade are somewhat reminiscent of the clinical observations of psychotic symptoms induced by LSD. Although the difference in species must be considered, our results suggest the possibility that sites of dopaminergic inhibition could be another primary area in the CNS affect by LSD.

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