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POTENTIATION OF APOMORPHINE-INDUCED CLIMBING BEHAVIOUR IN MICE BY d-LSD

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Abstract

1. The climbing behaviour in mice was used for studying possible interaction(s) of d-LSD with dopamine receptors.
2. Doses of d-LSD ranging from 0,25 to 2,5 mg/kg injected intra-peritoneally constantly inhibited the climbing behaviour.
3. In contrast, when similar doses of d-LSD were injected 10 min before apomorphine (5 mg/kg), a constant potentiation of the apomorphine-induced climbing was observed.
4. Subsequent experiments performed with a neuroleptic (haloperidol) or a serotonin precursor (5-OH-tryptophan) compared to those of d-LSD with and without apomorphine would indicate that d-LSD alone displays typical serotoninergic syndrome (including inhibition of the climbing), whereas in the presence of apomorphine, an interaction at presynaptic receptors may possibly modulate dopaminergic activity.

Keywords : apomorphine, d-LSD, serotonin, dopamine receptors, striatum, climbing behaviour.

Abbreviations : Apo : Apomorphine; c-AMP : cyclic AMP; d-LSD (or LSD) : d-lysergic acid diethylamide; 5-OH-T : 5-hydroxytryptophan.

Introduction

The circling behaviour induced by d-LSD in rats unilaterally injected in the medial forebrain bundle with 5,6-dihydroxytryptamine or 6-hydroxydopamine (Pieri et al. 1974) has been explained in terms of a direct stimulation of striatal dopamine receptors. However, such an effect of d-LSD in those behavioural studies has been debated and attributed to the fact that very high doses of the drug have been used (Pycock and Anlezark, 1975; see also the reply of Pieri et al. 1975).

In biochemical studies on the other hand, it has been shown that d-LSD was able to stimulate the formation of c-AMP in homogenates of the whole brain and in various brain areas (Bockaert et al. 1976; Da Prada et al. 1975; Spano et al. 1975) as well as in cerebral cortex and striatal crude mitochondrial fractions (Von Hungen et al. 1974, 1975a). Furthermore, d-LSD was a potent antagonist of the dopamine-elicited accumulation of c-AMP in the same preparations. Since it is not excluded that in striatum or in other brain structures d-LSD may interact with other monoamine receptors (such as noradrenaline and/or serotonin) (Aghajanian, 1972; Anden et al. 1968; Palmer and Burks, 1971; Von Hungen et al. 1975a, 1975b), we have decided to reevaluate some central

effects of d-LSD remaining controversial, such as a direct stimulation of dopamine receptors. For that purpose, we have used in the present study the climbing behaviour in mice (Costentin et al. 1975). Up to now, this behavioural test has never been used for unmasking potential dopamine-mimetic effects of d-LSD.

The purpose of the present study was to investigate the effects of d-LSD alone, or in combination with apomorphine, upon the climbing behaviour in mice.

Material and Methods

Experimental conditions

Animals. The experiments were performed on ICRZ strain albino male mice weighing 25-40 g (Institut für Zuchthygiene der Universität Zürich, Switzerland). The animals were maintained in the same conditions of housing (12h/12h light-dark cycle) and food and water were available ad libitum.

Drugs and solutions. The drugs used were d-LSD (Sandoz AG, Basle, Switzerland), apomorphine-HCl (Siegfried AG, Zofingue, Switzerland), 5-hydroxytryptophan (Calbiochem AG, Lucerne, Switzerland) and haloperidol (Cilag-Chemie AG, Schaffhouse, Switzerland). The solvents of drugs consisted of Ringer solution containing 1% ascorbic acid. Haloperidol was dissolved in a small amount (1/10 of the total volume) of 2-Ethoxyethanol before appropriate dilution with Ringer-ascorbic acid solution. All the drugs were injected intraperitoneally.

Experimental procedure

The technique of climbing behaviour used was similar to that reported by Costentin et al. (1975).

Apparatus. The cylindrical cages (12 cm diameter, 14 cm height) were built in our laboratory according to the description given by Protais et al. (1976). The walls were made with vertical metal bars (2 mm diameter) separated by a distance of 1 cm. Mice were introduced into the cages by opening the top sliding lids.

Procedure. Each animal was pushed into a cylindrical cage (Costentin et al. 1975) after receiving intraperitoneally 0,5 ml of Ringer containing 1% ascorbic acid (solvent) or appropriate dilutions of drugs in the same Ringer. When 2 or 3 drugs had to be injected, a time schedule and a rank order of injections were chosen depending upon the type of experiments (see Results). An adaptation time of 5 min was counted before scoring the climbing every 2 min up to 30 min according to the scale given by Costentin et al. (1975) : (0) four paws on the floor, (1) forefeet holding the vertical bars, (2) four paws holding the vertical bars. Means of scores (Ringer only) at a precise time of observation or for cumulated observations were compared to the means of scores (every 2 min up to 30 min) following drug injection, a rest period of 30 min being observed between the two treatments.

Study design. A set of three experiments have been performed :
- Experiment 1 : Effects of d-LSD upon spontaneous climbing behaviour
- Experiment 2 : Effects of d-LSD upon apomorphine-induced climbing behaviour
- Experiment 3 : Effects of d-LSD or 5-hydroxytryptophan upon apomorphine-induced climbing behaviour.

The detailed description of these experiments is given in Chapter "Results". The experimental design of the three above-mentioned experiments is given in Table 1.

Table 1

Experimental design of the three experiments

Exp. 1	Five groups of 6 mice			
	time 0 : solvent i.p.			
	" 15 to 45(min) : Climbing behaviour (no drugs)			
	" 45 to 75 : rest period (out of the cages)			
	" 75 : d-LSD i.p.			
	" 90 to 120 : climbing behaviour (d-LSD)			
Exp. 2	Group 1	Groups 2-5 (6 mice each)		
	time 0 : solvent i.p.	d-LSD i.p.		
	" 10 (min) : apo i.p.	apo i.p.		
	" 20 to 50 : climbing behaviour	climbing behaviour		
Exp. 3	Group 1	2	3	4(6 mice each)
	time 0 : 5-OH-T i.p.	-	-	5-OH-T
	" 20 (min) : -	d-LSD i.p.	solvent i.p.	-
	" 30 : apo i.p.	apo i.p.	apo i.p.	solvent i.p.
	" 35 to 65 : CB[a]	CB[a]	CB[a]	CB[a]

CB[a] = climbing behaviour

Statistical analysis. Statistical analysis was performed according to the Student-t-test for paired and/or unpaired data.

Results

Experiment 1 : *The effects of d-LSD upon climbing behaviour* (Fig. 1)

Five groups of six naïve mice were injected with 0,5 ml of solvent, pushed into individual cages and observed for the climbing behaviour 15 min after the injection (Fig. 1, white bars). After a 30 min rest (out of the cages), each group of mice received various doses of d-LSD (0,1 to 2,5 mg/kg) and a summation of scores (divided by the number of observations) obtained every 2 min, 15 to 45 min after drug injection is shown in Fig. 1 (hatched bars). Inhibition of climbing behaviour due to d-LSD is maximal 15 min after drug injection and the value remains more or less constant throughout 30 to 50 min (not shown).

Scores due to 0,25 to 2,5 mg/kg were significantly lower than those of control mice and they were dose-dependent (Fig. 1). It seems that d-LSD induces a typical behaviour which is opposite to the stimulation of climbing resulting from the activation of dopamine receptors by apomorphine (Costentin et al. 1975).

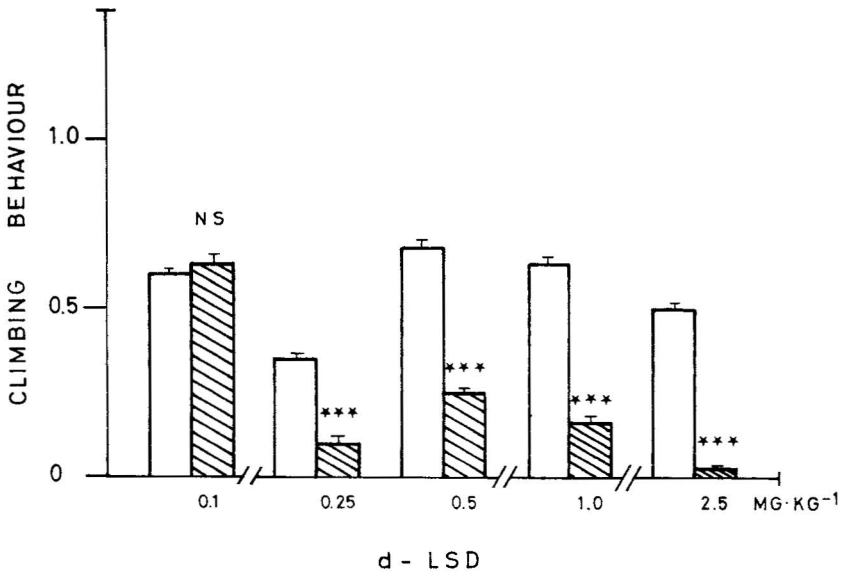

Fig. 1. Effect of increasing doses of d-LSD (0,1 to 2,5 mg/kg) upon spontaneous climbing behaviour in mice.
White columns : control animals (solvent); Hatched columns : d-LSD treated animals. NS = not significantly different from controls. *** $p < 0,0001$ (paired data)

Experiment 2 : *The effects of d-LSD upon apomorphine-induced climbing behaviour (Fig. 2)*

These experiments were performed on the same day with five groups of naïve mice. The first group received 0,5 ml of solvent. Ten minutes later, the animals were injected with 5 mg/kg of apomorphine, pushed into individual cages and scored every 2 min for climbing behaviour 10 min to 40 min after drug injection (Fig. 2, white column). Successively, four other groups of five mice were treated as for the first group with the exception that d-LSD doses ranging from 0,25 to 1,0 mg/kg were injected 10 min before apomorphine (Fig. 2, hatched columns). Climbing scores of d-LSD plus apomorphine treated mice were compared to the group of apomorphine treated mice (Fig. 2). Surprisingly, a potentiation of apomorphine-induced climbing behaviour was found in d-LSD treated mice with a maximal effect at 1 mg/kg.

Similar concentrations of d-LSD alone (Fig. 1) did not induce any climbing behaviour. On the contrary, d-LSD alone inhibits the normal behaviour of control mice.

Furthermore, the potentiation by d-LSD of the apomorphine effects seems to be specific for d-LSD (and not for dopamine receptor blocking agents) since haloperidol injected at 0,5 mg/kg in similar experimental conditions did not increase the scores due to apomorphine. In contrast, the observed scores in haloperidol plus apomorphine treated mice were close to 0 (not shown).

Experiment 3 : *The effects of d-LSD or 5-hydroxytryptophan upon apomorphine-induced climbing behaviour (Fig. 3)*

These experiments were performed on the same day with 4 groups of naïve mice. A first group of mice was injected with 5-0H-T (200 mg/kg) and left for 20 min. Then, the second and third groups of six naïve mice received 2,5 mg/kg of d-LSD or solvent, respectively. The three groups were injected 10 min later with 5 mg/kg of apomorphine, and each animal was pushed into individual cages. A fourth group of mice received only 5-0H-T (200 mg/kg) at time 0, as for the first group. The climbing behaviour was then observed 5 min after apomorphine injection (groups 1 to 3) and scored every 2 min for 30 min (including group 4).

Figure 3 shows the effect of d-LSD, 5-0Htryptophan and apomorphine alone. It could be seen that the scores of 5-0Htryptophan treated mice are comparable to those of d-LSD (see also Fig. 1).

No potentiation of apomorphine-induced climbing behaviour, but to some extent an inhibition, was observed with the serotonin precursor (5-0H-T). In contrast, d-LSD again (as in Fig. 2) was able to potentiate the apomorphine effect, inferring that this action of d-LSD, now in presence of apomorphine, was in some way related to the regulation of dopaminergic mechanism.

Interactions of d-LSD or newly formed serotonin (after 5-0H-T injection) with serotonin receptors were also expressed by several other typical behavioural effects such as resting tremor, hyperreactivity and hypertonus.

Discussion

Interactions of d-LSD with serotoninergic and/or dopaminergic neurones. Various biochemical and electrophysiological studies have shown that d-LSD can have marked effects on the serotonin containing cells as well as on the serotoninergic synapses. Furthermore, evidence exists that d-LSD can also interfere with central catecholaminergic systems (for review see Freedman and Halaris, 1978). More recently, several laboratories have shown that d-LSD can influence the monoamine- (including serotonin and dopamine) sensitive adenylate cyclase of various brain areas, by displaying agonist as well as antagonist properties (Nathanson, 1977). Finally, in binding studies of dopamine and/or serotonin receptors it has been found that d-LSD can be used as a stereo-specific radioligand or as a potent displacing agent (Burt et al. 1976). However, behavioural experiments performed by two groups of investigators raised a controversy as to a direct in vivo dopamine agonist effect of d-LSD (Pieri et al, 1974, 1975; Pycock and Anlezark, 1975). These and other behavioural data (Jacobs, 1976) led us to use the climbing behaviour in mice for investigating the mechanism of action of d-LSD. This model appears to be very selective for detecting short-term and/or long-term types of interaction of drugs with pre- and/or post-synaptic dopamine receptors (Costentin et al. 1975).

Interactions of d-LSD with apomorphine in climbing behaviour tests. The present paper reports the effects of d-LSD, and of a serotonin precursor (5-0H-T), administered alone and in association with apomorphine, upon the climbing behaviour in mice. The mode of administration of drugs (i.p.) and the range of doses injected by this way have been shown to produce various behavioural effects in mice or in other rodents (Di Chiara and Gessa, 1978; Fuxe et al. 1978;Horita and Hamilton, 1973; Jacobs, 1976). Doses of d-LSD inducing some significant effects upon the climbing behaviour (with or without apomorphine) were 10 to 25 times higher than those corresponding to hallucinatory-like behaviours (Jacobs et al. 1976). As previously shown by

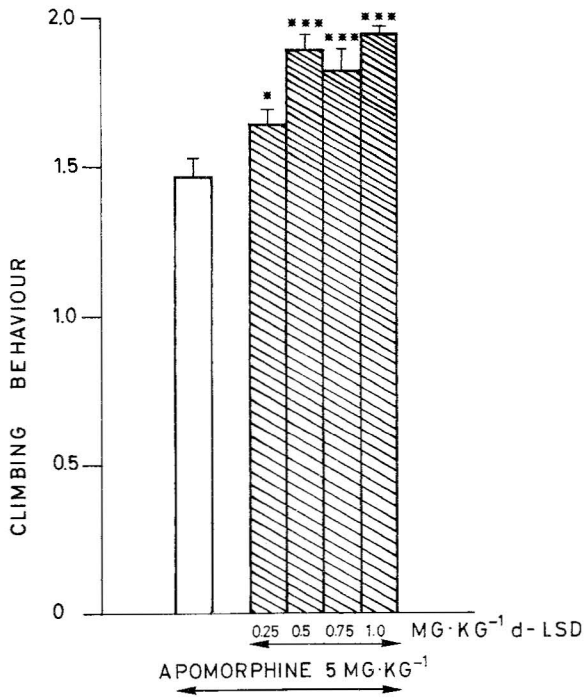

Fig. 2. Effect of increasing doses of d-LSD (0,25 to 1,0 mg/kg) upon apomorphine-induced climbing behaviour in mice. White columns : control apomorphine animals; Hatched columns : d-LSD apomorphine treated animals. *p <0,05; ***p <0,001 (unpaired data)

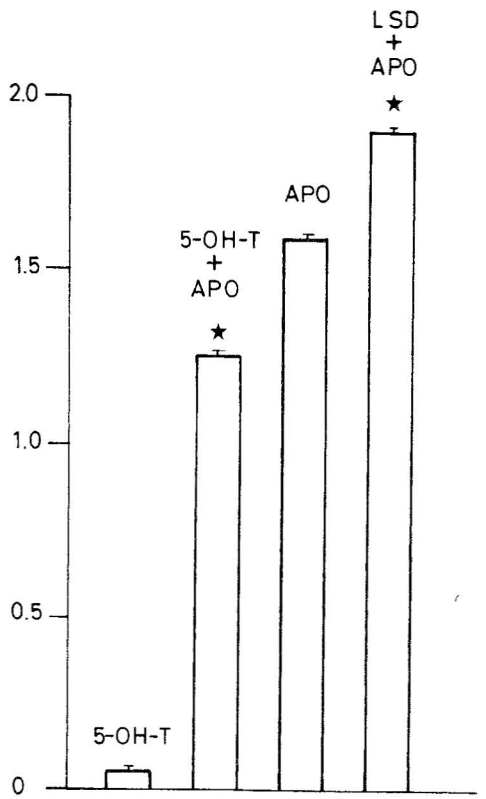

Fig. 3. Effect of d-LSD (2,5 mg/kg i.p.) and 5-hydroxytryptophan (200 mg/kg i.p.) upon apomorphine-induced climbing behaviour in mice. *p <0,01 compared with apomorphine alone (unpaired data)

others (Protais et al.1976) we did find that apomorphine at doses ranging between 0,5 and 5 mg/kg i.p. stimulated the climbing behaviour in mice as a result of the activation of postsynaptic striatal dopamine receptors (Costentin et al. 1975). In contrast, equivalent doses of d-LSD did produce a significant inhibition of the spontaneous climbing. Such an effect of d-LSD appears to be not mediated by dopaminergic mechanism(s) since the true serotonin precursor (5-hydroxytryptophan) induced the same inhibition of climbing behaviour. In addition, 5-hydroxytryptophan, as well as d-LSD, produced specific effects linked to central serotonin activity, such as arrest of exploring behaviour, resting tremor, hyperreactivity against exterior stimuli and hypertonus. Among other signs less frequently observed were reciprocal forepaw treading, wet dog shake behaviour, piloerection and hypersalivation. The serotonin syndrome has been shown to be produced by compounds which either

increase serotonin content such as 5-hydroxytryptophan or stimulate serotonin receptors such as d-LSD (Jacobs, 1976). Thus, d-LSD applied for climbing test in mice did not display apomorphine-like effects but rather a specific serotonin syndrome, as expected from other evidences obtained in various types of experiments (Freedman and Halaris, 1978; Jacobs, 1976). However, when both apomorphine and d-LSD were injected in a sequential manner (see Methods) the surprising finding was to detect a potentiation of apomorphine action by d-LSD. In contrast, 5-hydroxytryptophan did not potentiate the apomorphine stimulation of climbing. Rather the apomorphine action was to some extent inhibited by the serotonin precursor, which seems to imply a modulation by serotonin pathway(s) of the dopaminergic stimulation mediated by apomorphine (Moore et al., 1978). Furthermore, the potentiation of apomorphine effects by d-LSD cannot be attributed to a post-synaptic dopamine receptor-blockade, since haloperidol applied in similar experimental conditions as d-LSD completely inhibited the apomorphine-induced climbing and produced typical cataleptic syndrome (Bartholini, 1978).

Is one site of action of d-LSD located at striatal pre-synaptic dopamine receptors ? Our present explanation is related to a possible interaction of d-LSD with apomorphine at the pre-synaptic striatal receptors (autoreceptors). At the concentrations used, apomorphine is supposed to stimulate both populations of receptors (Baudry et al., 1977). Following stimulation of pre-synaptic receptors, the liberation of endogenous dopamine into the synaptic cleft is reduced, whereas direct stimulation of post-synaptic receptors is responsible for the climbing behaviour. Under d-LSD treatment, the apomorphine action is antagonised at pre-synaptic sites and more dopamine is released, which in turn would specifically stimulate post-synaptic receptors with concomitant potentiation of the climbing. Further biochemical and behavioural studies are now in progress in our laboratory, in relation with this working hypothesis and may bring about new insight into the target site(s) of action of d-LSD and/or other ergot alkaloid derivatives known to modulate dopaminergic mechanism(s).For review, see Kebabian and Calne (1979).

Conclusions

The present work indicates that d-LSD provokes typical effects linked to central serotonin activity in mice tested for climbing behaviour. In addition, d-LSD potentiates the climbing behaviour induced by apomorphine in a dose-dependent manner. The latter effect is not mimicked by classical dopamine receptor blockers such as haloperidol. It is therefore suggested that d-LSD can antagonize dopamine-mimetic drugs at striatal pre-synaptic receptors and may be considered as a modulator of some dopamine-mediated mechanisms.

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