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ACUTE AND CHRONIC EFFECTS OF LSD AND 5-MeODMT ON RAPHE-EVOKED DORSAL ROOT POTENTIALS IN THE CAT

Alice A. Larson

Department of Veterinary Biology, University of Minnesota
St. Paul, Minnesota 55108

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Summary

Both acute and chronic effects of lysergic acid diethylamide (LSD) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) on the dorsal root potential (DRP), evoked by stimulation of the nucleus raphe magnus of the cat, were examined. Single injections of LSD potentiated while those of 5-MeODMT inhibited the raphe-evoked DRP. The electrophysiologic response produced by each drug correlates well in dosage and time-course with their reported behavioral effects. Following four consecutive daily injections of LSD, complete tolerance developed to the potentiating effect of LSD on this potential. A similar pretreatment schedule with 5-MeODMT failed to alter its acute inhibitory effect on the DRP. These results correlate well with the development of tolerance to the behavioral effects of LSD and 5-MeODMT. This system may thus provide a unique electrophysiological model to examine the effects of these drugs.

We have previously shown that phasic electrical stimulation of the caudal raphe nuclei of the cat evokes depolarizing potentials which can be recorded from dorsal roots of the lumbar and sacral spinal cord (1, 2). A single thirty millisecond train of stimuli in the raphe evokes two negative dorsal root potentials (DRPs), designated DRP-1 and DRP-2, which are thought to reflect presynaptic inhibition of primary afferent fibers (1,2). While DRP-1 can be elicited from many sites in the brain stem (3, 4, 5), DRP-1 is only followed by the longer latency DRP-2 after stimulation at sites near the raphe nuclei (2). DRP-2 has been postulated to be mediated by 5-hydroxytryptamine (serotonin; 5-HT), based on the following evidence: 1) this potential is evoked only from brain stem areas containing serotonergic neurons which project to the spinal cord; 2) the long latency of DRP-2 is consistent with the slow conduction velocity expected for the small diameter serotonergic axons; and 3) DRP-2 is selectively blocked by several putative serotonin antagonists (1,2).

LSD has been postulated by many investigators to act, at least in part, by an interaction with the serotonergic system. We recently reported that low doses of LSD produced a gradual enhancement of DRP-2 over a 50 minute period (6) while higher doses of LSD, as well as all serotonin antagonists tested, selectively block this potential, with a maximal effect within 5 minutes of their i.v. injection (1, 2). Doses of LSD that enhance the area under DRP-2 fall into the range of doses that produce hallucinogenesis. In addition, the

time-course of LSD's low dose effect on DRP-2 also parallels the time-course of several behavioral effects which are thought to be related to hallucinogenesis (7).

It has been demonstrated that the discharge rate of certain serotonergic neurons decreases after systemic injections of low doses of LSD as well as after a variety of other hallucinogens (8, 9, 10). It is not known whether LSD inhibits the discharge rate of neurons specifically in the raphe magnus or obscurus. The onset of LSD's effect on discharge rate in the dorsal raphe nucleus, however, correlates with the inhibitory effect of low doses of LSD on DRP-2. Repeated injections of LSD, which is known to result in tolerance to both the hallucinogenic effect of LSD in man (11, 12, 13) as well as to the behavioral effects of LSD in animals (14-18), did not result in the development of tolerance to the inhibitory effect of LSD on raphe unit activity (19). Based on these findings, Trulson and Jacobs speculated that the development of tolerance to LSD is thus mediated by changes postsynaptic to the raphe neuronal system. This theory is supported by their finding that serotonin and LSD binding is altered by repeated injections of LSD (20).

DRP-2 is thought to reflect electrically evoked serotonergic activity in an area postsynaptic to the descending serotonergic system (6). The present study was designed to compare the effects of low doses of LSD to those of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) on the DRP-2, and to determine whether tolerance develops after pretreatment schedules which have been shown to cause tolerance to LSD but not to 5-MeODMT in a behavioral paradime (21, 22).

Methods

Cats were pretreated with atropine methyl nitrate (0.1 mg/kg) and anesthetized with ether. The brain rostral to the pons-medulla was rendered anoxic by using the anemic decerebration method of Pollock and Davis (23). Ether anesthesia was discontinued and the cats were artifically respired with room air after administration of gallamine triethiodide (Flaxedil) to immobilize the animals. Blood pressure was continuously monitored from a cannula placed in the carotid artery. Cats whose blood pressures dropped significantly during the experimental recording period were routinely discontinued as such changes have been found to alter the size of both DRP-1 and -2. Injections of 5-MeODMT, either i.m. or i.v., usually produced small, transiently decreases in blood pressure which did not appear to be of sufficient magnitude to affect the electrophysiological recordings, as DRP-1 was not attenuated.

After exposure of the spinal cord from the fourth lumbar to the second sacral segment, the seventh lumbar dorsal root was dissected free and mounted on platinum recording electrodes. A radiant heat source and a heating pad placed under the animal were electronically regulated to maintain the temperatures of the body and the mineral oil bath covering the cord at 37 $\pm$ 0.5°C.

All drugs and solutions were administered i.v. through an indwelling catheter placed in the antecubital vein. LSD was made up in saline while 5-MeODMT was dissolved initially in 0.1N hydrochloric acid and then diluted 1:10 (acid:saline) in saline. Injections of each vehicle alone, in volumes equal to those used for drug administration, did not affect DRP-1 or DRP-2. Gallamine triethiodide was purchased as an injectable solution. LSD was obtained from the National Institute of Mental Health (Bethesda, MD) and

5-Methoxy-N,N-dimethyltryptamine was purchased from Sigma Chemical Co. (St. Louis, MO). Cats pretreated with either LSD or 5-MeODMT were injected i.p. and i.m. respectively while in their home cages, and then prepared for DRP recordings 1 day after the last injection.

The raphe nuclei of the caudal brain stem were electrically stimulated by using a concentric bipolar stainless-steel electrode (model NE-100; David Kopf Instruments, Tujunga, CA), having an inner shaft diameter of 0.2 and 0.5 mm separation between the inner and outer barrel. The electrode was lowered into the brain stem using the stereotaxic coordinates of the inferior central nucleus (ICN) according to the atlas of Berman (24). The ICN of the cat includes the nucleus raphe magnus and nucleus raphe obscurus, both of which contain serotonergic cell bodies whose axonal processes project to the spinal cord in the cat (25) and rat (26).

Thirty millisecond trains of nine pulses (0.5 millisecond duration; 0.08-1.0 milliamps) were used to evoke each DRP. Recorded signals were amplified by a Tektronix 502 differential amplifier and viewed on a Tektronix 5103N oscilloscope. Sixteen consecutive DRPs elicited at 3-second intervals were summed by a Nicolet Model 527 Signal Averager and the resulting analog waveforms were plotted by using a Model 252A Cole-Parmer linear strip chart recorder. The waveform was divided into DRP-1 and DRP-2 at the time of the peak negative wave between the two potentials. The area beneath the waveforms was then measured using a Talos Cybergraph Digitizer interphased to a Hewlett Packard Model 9825 desk top computer. The areas under DRP-1 and -2 are routinely monitored for one hour following an i.v. saline injection to assure recording stability before drug administration.

Results

A single i.v. injection of 50 µg/kg of a 0.2 mg/ml solution of LSD, injected over a 15 sec period, elicited no immediate change in the area under either DRP-1 or DRP-2, but produced a significant enhancement of DRP-2 which developed slowly over a period of 60 to 90 minutes (Figure 1A and Table 1). DRP-1 remained unchanged over this time period. The photographs in figure 1A illustrate, by single sweeps on the oscilloscope, the acute effect of 50 g/kg of LSD in one cat, immediately before and 90 minutes after its injection.

In contrast to the acute effect of LSD, administration of 50 µg/kg of 5-MeODMT i.v. produced an immediate inhibition of DRP-2 which appeared maximal between just 3 and 5 minutes after its injection (Figure 1B and Table 1). 5-MeODMT was administered as a 0.25 mg/ml solution over a 90 sec time period. The photographs in figure 1B illustrate, by single sweeps on the oscilloscope, the effect of 50 µg/kg of 5-MeODMT immediately before and 3 minutes after its injection into an untreated control cat. DRP-2 completely recovered from the effect of 5-MeODMT by 30 minutes after its injection. Continous monitoring for at least 2 hours after the time of injection of 5-MeODMT indicated that neither DRP-1 nor DRP-2 were altered in magnitude after recovery from the initial effect of this drug on DRP-2. Injection of a lower dose of 5-MeODMT (10 g/kg i.v.) in 3 cats was found to have no effect on the amplitude of either DRP-1 or DRP-2 at any time during a 2 hours post-injection recording period.

Both DRP-1 and DRP-2 were successfully recorded from cats the day following the last day of pretreatment with either LSD or 5-MeODMT, as shown by the photographs of single oscilloscope tracings in Figure 2A and 2B respectively. After pretreatment of cats with 100 µg/kg of LSD i.p. per cat

daily for 4 days, a challenge injection of 50 µg/kg of LSD i.v. failed to enhance DRP-2 at any time after its injection (Figure 2A and Table 1). In contrast, the DRP-2 recorded from cats injected daily with 100 µg/kg of 5-MeODMT i.m. for 5 days were still significantly inhibited by an injection of 50 µg/kg of 5-MeODMT i.v. (Figure 2B and Table 1).

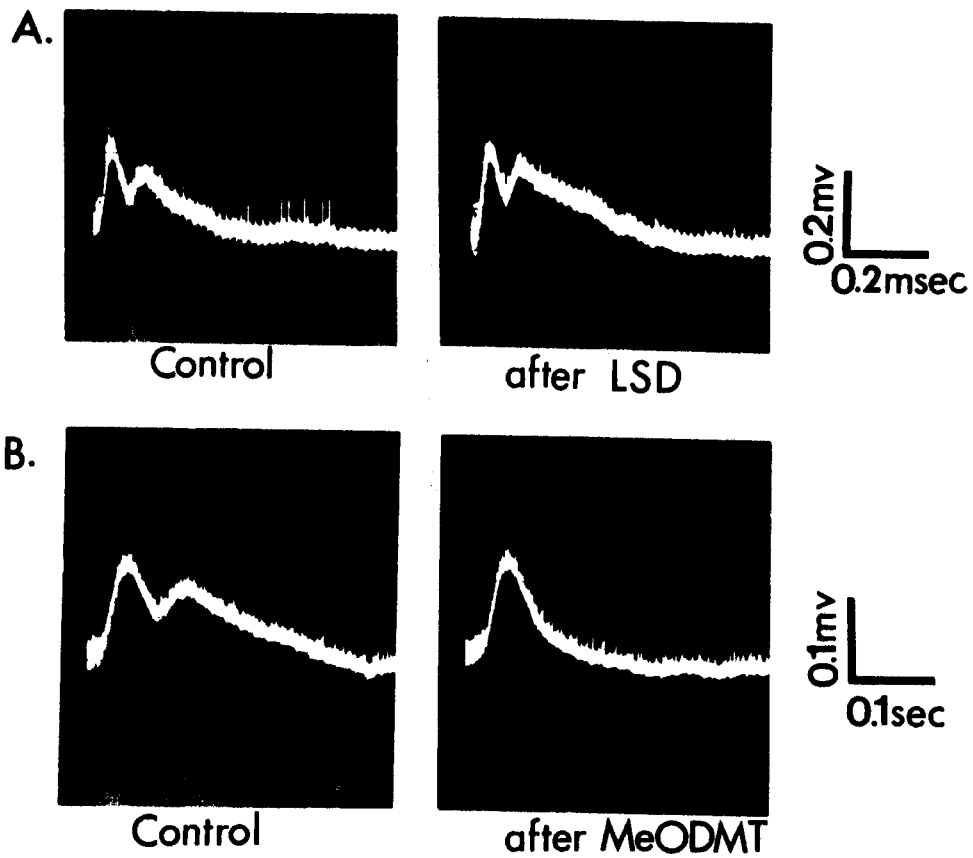

Fig. 1

Representative single sweeps on the oscilloscope showing acute effects of LSD and 5-MeODMT on DRP-1 and DRP-2. The first frame in A depicts DRP-1 and DRP-2 (indicated by 1 and 2) recorded from a control, non-treated cat immediately before an i.v. injection of 50 µg/kg of LSD, while the second frame shows enhancement of the area under DRP-2 at 90 minutes after this injection. The first photograph in B shows DRP-1 and DRP-2 just prior to the injection of 50 µg/kg of 5-MeODMT i.v., while the second frame shows the inhibition of DRP-2 at 3 minutes after this injection.

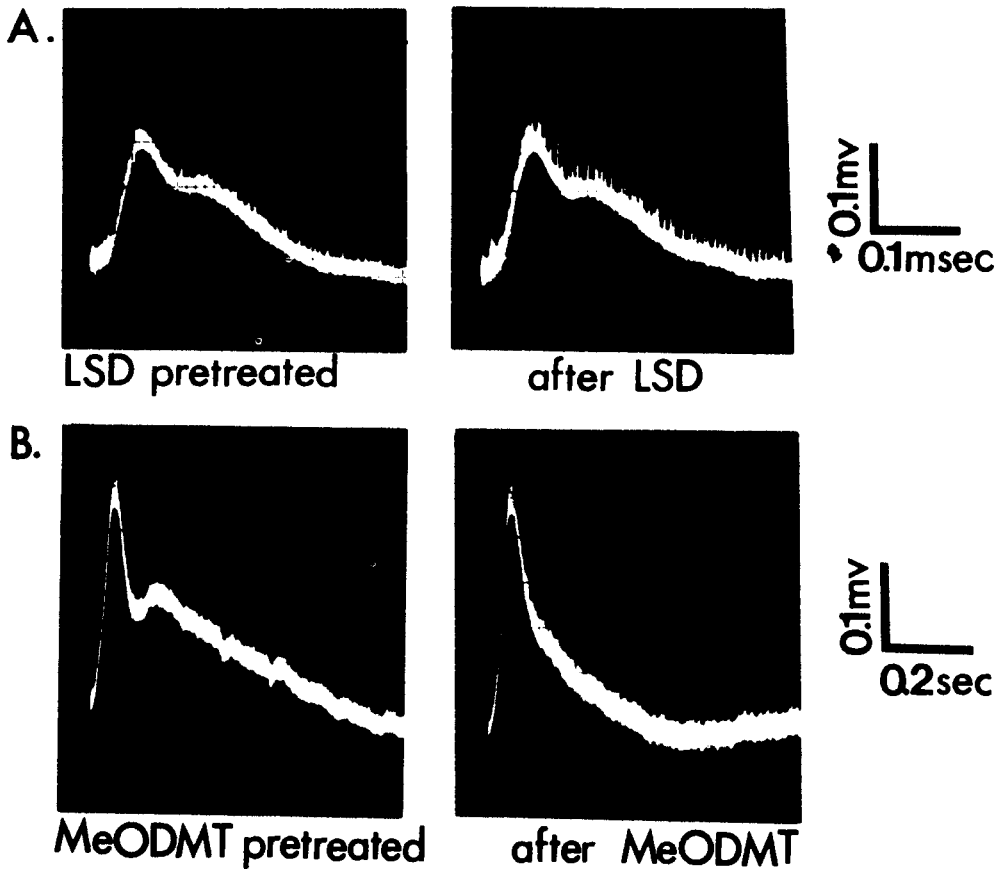

Fig. 2

Representative single sweeps on the oscilloscope showing tolerance to the effect of LSD but not to that of 5-MeODMT. The first photographs in A depicts DRP-1 and DRP-2 recorded from a cat that had been injected daily i.p. for 4 days with 100 µg/kg of LSD, while the second frame shows the lack of effect of 50 µg/kg of LSD i.v. in this cat at 90 minutes after its injection. The first photograph in B depicts DRP-1 and DRP-2 recorded from a cat that had been injected daily for 5 days with 100 µg/kg of 5-MeODMT i.m., while the second frame illustrates the ability of 50 µg/kg of 5-MeODMT i.v. to inhibit the DRP-2 in that same animal at 3 minutes after its injection.

TABLE I

Influence of LSD and 5-MeODMT on Raphe-Evoked DRP

Treatment+	Pretreatment	Percent of Control ++ Area Under DRP-2 S.E.M.	
LSD 50 µg/kg	-	148.0** + 20.9	(n = 6)
LSD 50 µg/kg	100 µg/kg/day for 4 days	108.5 + 7.7	(n = 4)
5-MeODMT 50 µg/kg	-	48.5* + 12.0	(n = 4)
5-MeODMT 50 µg/kg	100 µg/kg/day for 5 days	64.6* + 11.7	(n = 4)

Values in parentheses indicate number of cats in each group.

+ All drugs were administered i.v.

++ The area under DRP-2 was measured 3 minutes after injection of 5-MeODMT and 90 min after injection of LSD.

* Significantly different than the average control value obtained 3 minutes after treatment with saline only ($P<0.05$; Student's t-test).

** Significantly different than the average value obtained 90 minutes after treatment with saline only ($P<0.05$; Student's t-test).

Discussion

As previously reported, a single intravenous injection of 50 µg/kg of LSD produces a slowly developing enhancement of DRP-2 (6). This effect of low doses of LSD on DRP-2 correlates in dose-range and time-course with the ability of LSD to inhibit spontaneous raphe unit activity (27) and with the production by LSD of several behavioral phenomena which are thought to be related to hallucinogenesis (18, 28). LSD has also been found to potentiate the serotonin-induced facilitation of facial motorneurons (29) and facilitate flexor and C-fiber reflexes (30, 31). While the mechanism involved in the acute effect of LSD on DRP-2 is unknown, this effect of LSD on an evoked potential, as well as on certain behavioral parameters have been postulated by many investigators to be due to its action postsynaptic to the raphe neuron (6, 27).

LSD has been shown to interact with serotonergic receptors as an agonist as well as an antagonist (32). LSD's hallucinogenic activity, however, may

not result from such direct actions in light of the fact that serotonin agonists and antagonists do not consistently exhibit hallucinogenic activity. DRP-2 is inhibited by a wide variety of serotonin antagonists (1,2). Similarly, serotonin agonists would be expected to result in a tonic depolarization of primary afferent terminals, occluding the phasically evoked DRP-2. Such an occlusive effect is seen after treatment with 25 mg/kg 5-hydroxytryptophan. Thus inhibition of DRP-2 by high doses of LSD may reflect either an agonistic or antagonistic interaction of LSD with the serotonergic receptor.

The enhancement of DRP-2 by low doses of LSD cannot be explained in terms of such direct interactions of LSD with serotonergic receptors, although LSD-induced changes in serotonin-receptor binding may play a role in the development of tolerance to LSD. While a single injection of LSD did not produce a significant change in (^{3}H)-serotonin binding, chronic administration of LSD, using similar doses and injection schedule as described in the present studies, resulted in a decreased maximal (^{3}H)-serotonin and (^{3}H)-LSD specific receptor binding (20). The development of tolerance to the ability of LSD to potentiate DRP-2 may thus reflect such an altered binding.

Early observations by Krivoy describe an enhanced accumulation of Substance P after an acute injection of LSD in mice both *in vitro* (33) and *in vivo* (34). Such an accumulation of Substance P by LSD would explain the ability of LSD to enhance C-fiber reflex activity in the dog spinal cord, as reported by Bell and Martin (32). In light of the findings by Hokfelt *et al* (35) that substance P is co-localized in descending serotonergic neurons, an enhanced accumulation of Substance P by LSD may be of pharmacological importance in the regulation of serotonergic activity. For example, Mitchell and Fleetwood-Walker (36) have shown that Substance P may play a role in the regulation of serotonin release from raphe neurons by interacting with presynaptic serotonergic auto receptors, thus attenuating feedback inhibition. An enhanced accumulation of Substance P produced by an acute injection of LSD would then act to enhance the release of serotonin, thereby potentiating the magnitude of the DRP-2.

In contrast to the facilitating effect of low doses of LSD on DRP-2, a single injection of 5-MeODMT produced an immediate inhibition of this potential. The effect of single injections of 5-MeODMT on DRP-2 were found to be inhibitory at doses from 10 μg/kg i.v. (n = 3 cats) to 1 mg/kg i.m. (n = 2 cats). Although a wide variety of serotonin antagonists, including LSD, have been reported to inhibit DRP-2 (1, 2), 5-MeODMT is thought to interact at the serotonergic receptor as an agonist rather than an antagonist (37, 38). We would therefore postulate that the inhibition of DRP-2 by 5-MeODMT results from such agonistic activity, causing a temporary, tonic occlusion of the phasically evoked DRP-2.

The failure of chronic 5-MeODMT treatment to produce tolerance to its inhibitory effect on DRP-2 following the chronic injection schedule described in this study, correlates well with the lack of tolerance to either its behavioral effects (10) or to its inhibition of raphe neuronal firing (10, 39). However chronic administration of 5-MeODMT to rats using a more frequent injection schedule (2 mg/kg i.p. every 30 minutes for 4 hours) has been found to produce tolerance (40) to its ability to induce various symptoms of the "serotonin syndrome" (see review of this syndrome by Jacobs 41).

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