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Action of Hallucinogens on Raphe-Evoked Dorsal Root Potentials (DRPs) in the Cat[1]

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LARSON, A A AND E G ANDERSON *Action of hallucinogens on raphe-evoked dorsal root potentials (DRPs) in the cat* PHARMACOL BIOCHEM BEHAV **24**(2) 347–350, 1986 —The dorsal root potential (DRP) evoked by stimulation of the inferior central nucleus (ICN) of the cat is affected by administration of a variety of hallucinogenic agents It has been previously shown that a single low dose of LSD is unique in that it potentiates this DRP, while injections of 5-methoxy-N,N- dimethyltryptamine (5-MeODMT), ketamine or phencyclidine (PCP) inhibit its production Tolerance develops to the facilitatory effect of low doses of LSD on the DRP, but not to the inhibitory action of 5-MeODMT Repeated injections of ketamine every 30 minutes also fail to produce tachyphylaxis to the inhibitory effect of this dissociative anesthetic The raphe-evoked DRP is a long latency potential that is inhibited by a wide variety of putative serotonin antagonists and has therefore been traditionally thought to be mediated by serotonin However, in light of the inability of either tryptophan or fluoxetine to potentiate this DRP, and the resistance of this DRP to blockade by parachlorophenylalanine, reserpine or intrathecally administered 5,7-dihydroxytryptamine, it appears that this potential may in fact be mediated, at least in part, by a non-serotonergic transmitter

Hallucinogens Serotonin LSD Dorsal root potentials

WE have previously shown that phasic electrical stimulation of the inferior central nucleus (ICN) of the cat evokes depolarizing potentials which can be recorded from dorsal roots of the lumbar and sacral spinal cord [22,23] A single thirty millisecond train of stimuli in this area of 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 [22,23] Changes in this primary afferent depolarization may affect somatic sensations and thus play a role in hallucinogenesis While DRP-1 can be elicited from many sites in the brain stem [5, 17, 21], DRP-2 is evoked only following stimulation at sites near the raphe nucleus [23]

Lysergic acid diethylamide (LSD), 5-methoxy-N,N- dimethyltryptamine (5-MeODMT) and the dissociative anesthetics ketamine and phencyclidine (PCP) have been postulated by many investigators to act, at least in part, by an interaction with the serotonergic system We have previously shown that these hallucinogens all affect the DRP-2 A single injection of a low dose of LSD is unique in that it potentiates DRP-2 [16] while this potential is selectively inhibited by 5-MeODMT [14], ketamine and PCP [15]

DRP-2 has been postulated to be mediated by 5-hydroxytryptamine (serotonin, 5-HT), based on the evidence that (1) DRP-2 is evoked only from brain stem areas containing serotonergic neurons which project to the spinal cord, (2) the DRP-2 is a long latency potential, consistent with the slow conduction velocity expected for the small diameter serotonergic axons, and (3) DRP-2 is selectively inhibited by several putative serotonin antagonists [22,23] Although serotonin antagonists are potentially useful tools in the study of serotonergic neurons in the brain, a major limitation in the use of these agents has been the controversy in the literature over the specificity and effectiveness of these drugs when used in the central nervous system (CNS) [9]

In order to interpret the unique actions of the hallucinogenic compounds which have previously been reported to affect DRP-2, it is necessary to more extensively evaluate the role of serotonin as the mediator involved in the elicitation of DRP-2 The present study summarizes the effects on DRP-2 of a variety of compounds known to alter serotonergic activity in the CNS and the implications of these results on the actions of hallucinogens on this electrical response

METHOD

Surgical Preparation

Cats weighing between 2 to 4 kg were pretreated with atropine methyl nitrate (0 1 mg/kg) and anesthetized with ether The brain, rostral to the pons-medulla, was rendered anoxic using the anaemic decerebration method of Pollock and Davis [20] Ether anesthesia was discontinued and the cats were artificially respired with room air Gallamine

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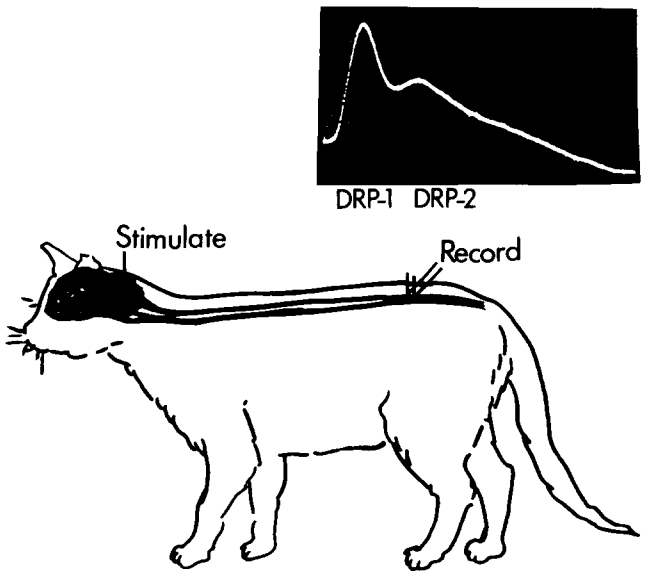

FIG 1 Electrical stimulation of the ICN of the cat elicits two potentials, DRP-1 and DRP-2, which can be recorded extracellularly in dorsal roots of the lumbosacral area of the spinal cord Activation of these descending pathways is thought to alter somatic sensations by presynaptic inhibition of primary afferent fibers

triethiodide (flaxedil) was administered periodically to immobilize the animals Blood pressure was continuously monitored from a cannula placed in the carotid artery

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 source of radiant heat was electronically regulated to maintain the temperature of the mineral oil bath covering the cord at 37±0 5°C

Drug Administration

All drugs and solutions administered intravenously (IV) were injected via an indwelling catheter placed in the antecubital vein Gallamine triethiodide was purchased as an injectable solution (Flaxedil, Lederle Pharmaceuticals, Carolina, Puerto Rico) Racemic ketamine hydrochloride was purchased as an injectable solution (Ketaset, Bristol, Syracuse, NY) and diluted with saline Phencyclidine hydrochloride (PCP) and lysergic acid diethylamide (LSD), gifts from the National Institute on Drug Abuse, were dissolved in saline 5-Methoxy-N,N-dimethyltryptamine (5-MeODMT), L-tryptophan, DL-para-chlorophenylalanine (PCPA), reserpine and 5,7-dihydroxytryptamine creatinine sulfate were purchased from Sigma Chemical Co (St Louis, MO) 5-MeODMT and tryptophan were dissolved initially in 0 1 N hydrochloric acid and then diluted 1 10 (acid saline) in saline Injections of this vehicle alone, in volumes equal to those used for drug administration, did not alter DRP-1 or DRP-2 PCPA was prepared by mixing with 0 5 g pectin for each intraperitoneal (IP) injection Reserpine was initially dissolved in propylene glycol and then diluted with saline for IP injection 5,7-Dihydroxytryptamine was dissolved in 0 01% ascorbic acid saline and administered intrathecally (IT) via an indwelling cannula which was implanted by the method described by Taksh and Rudy [27] at least 5 days prior to the injection The tip of the cannula projected to the level of the lumbar area of the cord Fluoxetine hydrochloride, a gift from Lilly (Indianapolis, IN) was dissolved in saline All drug doses are expressed in terms of the salt

Electrical Stimulation and Recording

The raphe nuclei of the caudal brain stem were electrically stimulated using a concentric bipolar stainless steel electrode (Kopf, model NE-100) 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 [4] 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 [3]

Thirty millisecond trains of nine pulses (0 5 msec duration, 0 08–1 0 mA) were used to evoke DRP Recorded signals were amplified by a Tektronix 502 differential amplifier and viewed on a Tektronix 5103N oscilloscope (Fig 1) Sixteen consecutive DRPs elicited at 3 sec intervals were totalled by a Nicolet Model 527 Signal Averager and the resulting analog waveforms were plotted using a linear strip chart recorder The area beneath the waveforms was measured using a Talos Cybergraph Digitizer interfaced to a Hewlett-Packard Model 9825 desk-top computer

RESULTS AND DISCUSSION

The effects of a variety of hallucinogenic compounds on DRP-2 are summarized in Table 1 As previously reported, high doses of LSD, ranging from 0 1 to 1 mg/kg IV, produce an immediate inhibition of DRP-2 which is accompanied by a simultaneous enhancement of DRP-1 Both potentials recover by approximately 30 minutes These effects of LSD are identical to those elicited by the serotonin antagonists methysergide, cinanserin and 2-bromo-D-lysergic acid diethylamide bitartrate (BOL) as reported previously [22,23] In contrast, a lower dose of LSD, or low doses of BOL and methysergide, elicit little or no immediate change in the magnitude of either DRP-1 or DRP-2, but instead produce a selective enhancement of DRP-2 which develops slowly over a 90 minute time interval (Table 1) Of the three drugs found to enhance DRP-2 in this fashion, LSD proved by far the most efficacious

On the other hand, the administration of 5-MeODMT, ketamine or PCP each produces an immediate inhibition of DRP-2 (Table 1) The effect of these drugs differs from that of the serotonin antagonists in that the inhibition of DRP-2 by the dissociative anesthetics is not accompanied by any change in the magnitude of DRP-1 [14,15] It is unlikely that the inhibition of DRP-2 by the dissociative anesthetics is related to their hallucinogenic activity as the (+) isomer, which has been found to have less hallucinogenic activity than the (−) isomer, produces a greater decrease in DRP-2 than the (−) isomer [15] However, the selective depression of DRP-2 by the dissociative anesthetics does correlate well in time course and dose-range with their sedative anesthetic effects in cats Although general anesthetics like ether and the barbiturate thiamylal also inhibit DRP-2, DRP-1, and the segmentally evoked DRP, the dissociative anesthetics differ from these general depressants by their selective inhibition of DRP-2 and not DRP-1 [15] We have recently found the segmentally evoked DRP is also insensitive to the effect of ketamine

TABLE 1
INFLUENCE OF HALLUCINOGENS OF DRP-2

Treatment*	Percent of Control Area† Under DRP-2 ± S E	Number of Cats
LSD (0 1 mg/kg)	46 5 ± 8 7	5
LSD (0 05 mg/kg)	148 0 ± 20 9	6
5-MeODMT (0 05 mg/kg)	48 5 ± 12 0	4
Ketamine (1 mg/kg)	38 5 ± 8 9	7
PCP (0 5 mg/kg)	33 0 ± 5 3	3

*All drugs were administered IV
†The area under DRP-2 was measured 2–3 min after injection of 5-MeODMT, ketamine, PCP and the high dose of LSD and 90 min after the injection of 0 05 mg/kg of LSD

In addition to the contrasting effects on DRP-2, LSD and 5-MeODMT also differ in the tendency for tolerance to develop to their actions on DRP-2 Both DRP-1 and DRP-2 are successfully evoked from cats pretreated daily with 0 1 mg/kg of either LSD administered IP for 4 days or 5-MeODMT administered IM for 5 days Pretreatment with 5-MeODMT did not alter the inhibitory effect of 5-MeODMT on DRP-2, while pretreatment with LSD resulted in complete tolerance to the ability of LSD to enhance DRP-2 [14] This correlates well with the lack of tolerance to 5-MeODMT to either its behavioral effects [25] or to its inhibition of raphe neuronal firing [18,25] when injected in a similar pretreatment schedule A more frequent injection schedule is necessary for the production of tolerance to this drug, as demonstrated by Keltch *et al* [11]

The production of tolerance to the facilitatory effect of LSD on DRP-2 correlates well with tolerance development to both the hallucinogenic effect in man [1, 6, 10] and to the behavioral effect of LSD in animals [2, 7, 8, 24, 26] The DRP-2 may thus provide a unique electrophysiological model to examine the effects of this drug It is of interest to determine with certainty the mediators involved in the production of this potential

Considering the accumulation of evidence for its involvement in both acute and chronic effects of LSD, as well as its localization in the descending bulbospinal pathway, serotonin is, of course, a logical candidate To further test the hypothesis that serotonin is a mediator of DRP-2, we administered a number of pharmacologic agents which have been reported to alter serotonergic activity

Injection of 5 and 10 mg/kg of IV fluoxetine, a serotonin reuptake inhibitor, failed to significantly alter the magnitude of either DRP-1 or DRP-2 L-tryptophan, the amino acid precursor of serotonin, also failed to affect either potential when administered in doses of 25 to 50 mg/kg IV Injections of 50 and 100 mg/kg of PCPA methyl ester HCl in saline were also without effect on these potentials over a 2 hour time interval after their IV injection

Because PCPA may require several days to deplete serotonin, 3 cats were pretreated with 300 mg/kg of PCPA at 24 and 48 hours prior to electrophysiological recordings Both DRP-1 and DRP-2 were successfully elicited in these animals Similarly, pretreatment with 0 5 mg/kg of reserpine IP 24 hours prior to the experiment did not affect our ability to elicit a DRP-2 in 4 out of 5 cats tested Injection of 50 mg/kg IV of PCPA methyl ester in 4 cats pretreated with reserpine not only failed to inhibit DRP-2, but slightly enhanced the DRP-2 recorded in one cat

Cats were implanted with intrathecal (IT) cannulas allowing injections directly into the spinal subarachnoid space Seven cats were injected IT with 0 8 mg of 5,7-DHT one week prior to recording and three additional cats were injected with the same dose 6 and 7 days prior to the experiment Both DRP-1 and DRP-2 were successfully recorded in these animals Injection of 0 05 mg/kg of LSD IV into 4 of these animals produced a potentiation of DRP-2 which was not significantly different than that obtained in non-pretreated animals

Together these data would indicate that while DRP-2 is very sensitive to inhibition by serotonin antagonists, it appears to be remarkably resistant to other pharmacologic manipulations of the serotonergic system One might argue that any one of these drugs may not have either blocked or potentiated DRP-2 due to inadequate dosage resulting in, for example, inadequate depletion of serotonin to affect the DRP-2 However, the wide variety of agents used to manipulate the serotonergic system, together with the lack of specificity of serotonin antagonists, would strongly argue against the mediation of this potential by serotonin The possibility that serotonin acts to modulate the DRP-2 under certain circumstances cannot be excluded

Based on the coexistence of other neuropeptides, for example substance P, in fibers descending from the raphe nuclei, it is possible that endogenous peptides or other substances may play a role in the production of this potential Mitchell and Fleetwood-Walker [19] have shown that substance P may play a role in the regulation of serotonin release from raphe neurons by interaction with presynaptic serotonergic autoreceptors, thus attenuating feedback inhibition Early observations by Krivoy describe an enhanced accumulation of substance P after an acute injection of LSD in mice both in vitro [12] and in vivo [13] Such an accumulation of substance P or other neuropeptides in the descending bulbospinal pathway would explain the ability of LSD to potentiate DRP-2 This is the topic of further investigations in this area

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