European Journal of Pharmacology, 48 (1978) 431—437

SINGLE DOSE AND CROSS TOLERANCE STUDIES OF β-PHENETHYLAMINE, d-AMPHETAMINE AND LSD IN THE CHRONIC SPINAL DOG †,*

D. BRUCE VAUPEL, MASAKO NOZAKI **, WILLIAM R. MARTIN *** and LON D. BRIGHT

National Institute on Drug Abuse, Division of Research, Addiction Research Center, Lexington, Kentucky 40583, U.S.A.

Received 9 August 1977, revised MS received 29 December 1977, accepted 6 January 1978

D.B. VAUPEL, M. NOZAKI, W.R. MARTIN and L.D. BRIGHT, *Single dose and cross tolerance studies of β-phenethylamine, d-amphetamine and LSD in the chronic spinal dog*, European J. Pharmacol. 48 (1978) 431—437.

The effects of β-phenethylamine (PEA), d-amphetamine and LSD were studied on spinal reflexes, autonomic signs, and behavior in the non-tolerant and LSD tolerant (30 μg/kg/day) chronic spinal dog. LSD (10 μg/kg) facilitated the flexor reflex, produced the stepping reflex, increased respiration, pulse rate and temperature, slightly dilated pupils and produced whining, tracking and restlessness. Direct tolerance developed to all of these effects except temperature. PEA (0.8 mg/kg/min infused for 12 min) and amphetamine (3.2 mg/kg) facilitated the flexor reflex, increased respiration, temperature and the skin twitch reflex latency, caused a marked mydriasis, retracted the nictitating membrane and produced restlessness and stereotypic head movements. PEA but not amphetamine elicited stepping, and only amphetamine consistently slowed heart rate. No cross tolerance to the physiologic or behavioral effects of amphetamine was observed. Partial tolerance developed only to the actions of PEA on the stepping reflex and the nictitating membrane. The single dose effects and the lack of cross tolerance to amphetamine and PEA suggest modes of action different from LSD. PEA has some actions which differ from those of amphetamine.

Tolerance β-Phenethylamine LSD Amphetamine Behavior

1. Introduction

The demonstration of β-phenethylamine (PEA) in the central nervous system (Durden et al., 1973; Inwang et al., 1973; Willner et al., 1974; Saavedra, 1974) and its central nervous system activity (Mantegazza and Riva, 1963; Sabelli et al., 1975; Martin and Eades, 1974) raise the possibility that it may not only have a physiologic role but could play a role in psychopathology (Sabelli et al., 1974; Martin et al., 1978). In studies of PEA in the chronic spinal dog, it was observed that it produced many effects similar to LSD which included mydriasis, tachypnea, facilitation of the flexor reflex and evocation of the stepping reflex (Martin and Eades, 1974). However, PEA could clearly be distinguished from LSD in that its effects were not antagonized by cyproheptadine, whereas those of LSD were. The relevance of PEA to addiction processes acquired additional significance when it was observed that PEA was reinforcing in the dog (Risner and Jones, 1977). The present study was conducted to further char-

† Under the auspices of U.S. Department of Health, Education and Welfare, Public Health Service, Alcohol, Drug Abuse and Mental Health Administration.
* A portion of this material was presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics, August 15—19, 1976 (Pharmacologist 18, 128, 1976).
** Present address: Department of Pharmacology, Cornell University Medical College, New York, N.Y. 10021, U.S.A.
*** Present address: Department of Pharmacology, University of Kentucky College of Medicine, Lexington, Kentucky 40506, U.S.A.

acterize the actions of PEA using the nontolerant and LSD tolerant chronic spinal dog. In conjuction with these experiments, LSD and d-amphetabmine were used as prototypic drugs with which PEA and other drugs have been compared.

2. Materials and methods

2.1. General procedures

The methodology has been previously described in detail (Martin and Eades,

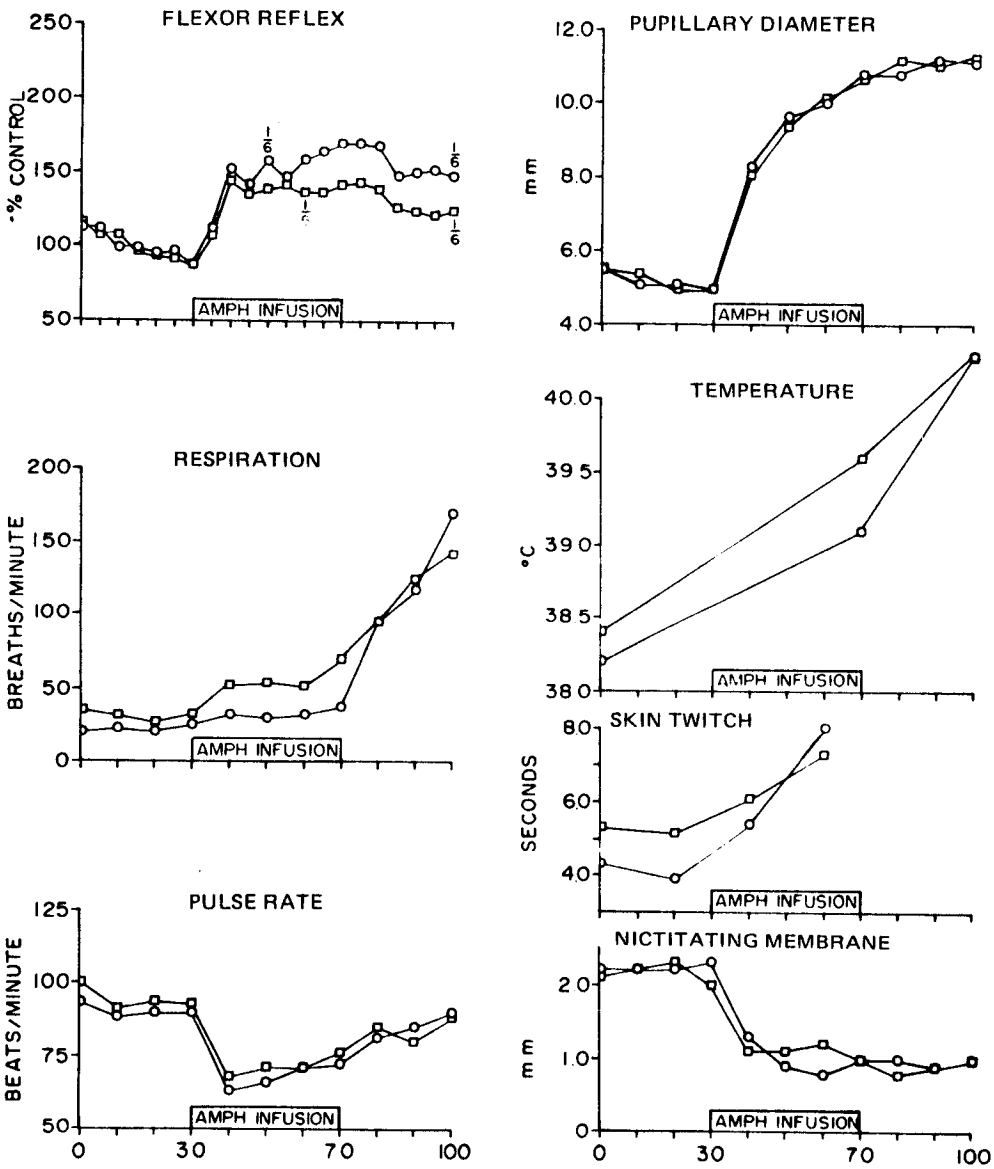

Fig. 1. Demonstration of the lack of cross tolerance to d-amphetamine (AMPH, 3.2 mg/kg) in dogs receiving 30 μg/kg/day of LSD. In the flexor reflex panel the proportion of dogs exhibiting the stepping reflex at a particular time is indicated by the fraction. ○———○, Pretolerance controls (n = 6); □———□, LSD tolerance (n = 6). Abscissae: time (min).

1967, 1970; Vaupel et al., 1977). In brief, 6 female beagle-type dogs whose spinal cords were transected at the T-10 or T-11 level were used in a Latin square design in which 6 drugs were administered i.v. at weekly intervals to obtain pretolerant measurements. The 6 drugs were LSD (10 μg/kg), d-amphetamine (3.2 mg/kg), β-phenethylamine (9.6 mg/kg), 2,5-dimethoxyamphetamine (2.4 mg/kg), 3,4-methylenedioxyamphetamine (2.0 mg/kg) and saline. The results obtained for 2,5-dimethoxyamphetamine and 3,4-methylenedioxyamphetamine have been published elsewhere (Vaupel et al., 1977; Nozaki et al., 1977). Jugular vein catheters were then surgically implanted, and the dogs were made gradually tolerant to 30 μg/kg/day of LSD over 6 days using a regimen of 6 daily programmed infusions (Vaupel et al., 1977). After establishing that tolerance to LSD was present by administering a 10 μg/kg challenge dose of LSD, all 6 drugs were again

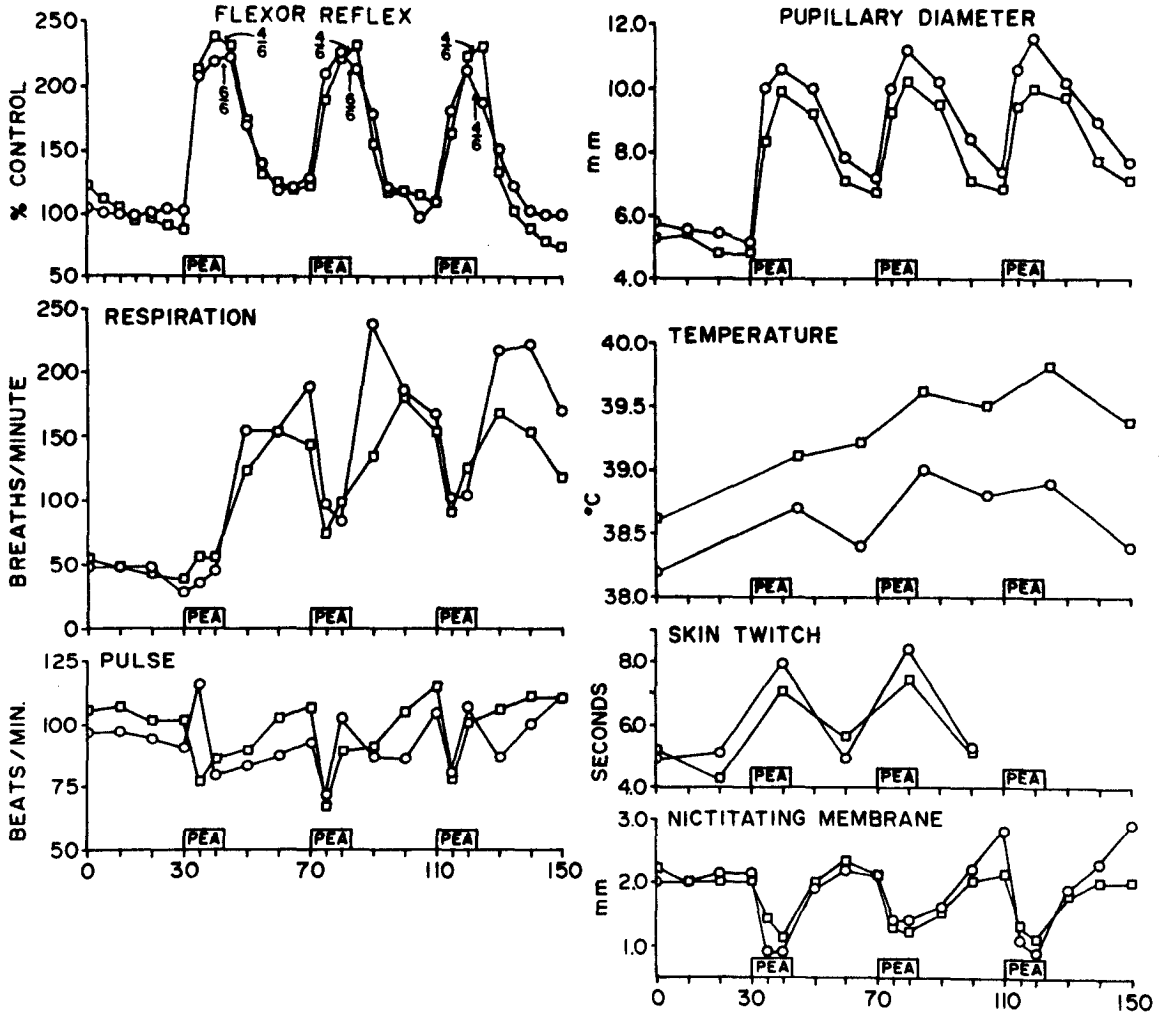

Fig. 2. Demonstration of the predominant lack of cross tolerance to β-phenethylamine (PEA, 9.6 mg/kg/12 min infusion) in dogs receiving 30 μg/kg/day of LSD. In the flexor reflex panel the proportion of dogs exhibiting the stepping reflex at a particular time is indicated by the fraction. o———o, Pretolerance controls (n = 6); □———□, LSD tolerance (n = 6). Abscissae: time (min).

given at 4 day intervals using a Latin square design. Measurements were made on the electrically evoked flexor reflex, the stepping reflex, respiration, heart rate, pupillary diameter, nictitating membrane width, the skin twitch reflex and rectal temperature. The general behavior of the dogs was rated as unresponsive, sleeping, quiet, restless or struggling and more specific signs such as vocalizations, stereotypic head movements and eye movements were scored as present or not (Vaupel et al., 1977; Nozaki et al., 1977).

Two protocols were employed: for LSD and amphetamine a 30 min control period was followed by a 40 min drug infusion and a 30 min postinfusion period (figs. 1 and 3). For β-phenethylamine, which is rapidly metabolized, a series of three infusions (9.6 mg/kg/12 min) was used. Following a 30 min control period, the infusions were spaced at 40 min intervals, and observations were continued for 30 min after the last infusion (fig. 2). The drugs were dissolved in saline and were infused intravenously at a rate of 0.5 ml/min. Dose levels were selected that were approximately equifacilitatory on the flexor reflex. Except for the stepping reflex onset latencies, temperature differences and respiration, areas under the time action curves during the respective drug infusions were calculated and compared using paired *t*-tests. Because the drug effects on respiration peaked after the infusions, areas were calculated from the 30th to 100th min for LSD and amphetamine and from the 30th to 70th min for β-phenethylamine. Latin square analyses were used to check for time-related changes during the pretolerance and LSD tolerance blocks. Nonparametric behavior data were analyzed using a χ^2 analysis (Vaupel et al., 1977; Nozaki et al., 1977).

3. Results

3.1. LSD

The infusion of LSD is characterized by a marked facilitation of the flexor reflex, the appearance of the stepping reflex, increased respiration and relatively small increases in pulse rate, pupillary diameter and body temperature (table 1). In this experiment, LSD did not increase the latency of the skin twitch reflex as it has in other studies (Vaupel et al., 1977). Behavioral effects of LSD included the development of restlessness (fig. 3), whining and tracking movements. The same spinal dogs receiving 30 μg/kg/day of LSD developed direct tolerance to all of the significant physiologic actions of LSD except for temperature (table 1) as well as to its behavioral effects (fig. 3).

3.2. d-Amphetamine

Doses of amphetamine (3.2 mg/kg) equieffective to LSD in facilitating the flexor reflex produced a bradycardia, retraction of the nictitating membrane, mydriasis, hyperthermia, an increase in respiratory rate and a prolongation of the skin twitch reflex latency (fig. 1; table 1). However, amphetamine did not elicit a well-developed stepping reflex. The restless behavior induced by amphetamine (fig. 3) was characterized by stereotypic head tossing, head rocking or head rotation, darting eye movements and almost no vocalization. Significant levels of cross tolerance could not be demonstrated for any of the physiologic or behavioral effects of amphetamine given to the LSD tolerant dog (figs. 1 and 3; table 1). Fig. 1 shows that in the 30 min following the amphetamine infusion, the facilitation of the flexor reflex in LSD tolerant dogs was less than the pretolerant response. However, the difference was not statistically significant. The experiment was repeated using 2 mg/kg of amphetamine to avoid the severe hyperthermia produced by the higher dose. Again, no cross tolerance developed to any of the actions of amphetamine, and the flexor reflex response in these experiments was slightly greater in LSD tolerant dogs than in untreated dogs. Thus the LSD tolerant dogs do not develop cross tolerance to d-amphetamine.

TABLE 1

Tolerance—cross tolerance study.

Responses to saline (SAL), LSD (lysergic acid diethylamide), AMPH (d-amphetamine) and PEA (β-phenethylamine) during the two treatment conditions are shown as the mean ± S.E. and represent area scores except for the stepping reflex and temperature. For saline, LSD and AMPH the maximum time limit allotted for the development of the stepping reflex is 40 min (i.e., 40 ± 0 min = No stepping). For PEA this value is 12 min. Statistical comparisons were made with one-tail paired *t*-tests. Control responses to LSD, AMPH and PEA in the non-tolerant dog were compared to SAL, and the level of statistical significance is shown by the superscript above the mean. The actual SAL control values for the 12 min PEA infusion are not shown. Direct tolerance to LSD and cross tolerance to AMPH and PEA were assessed by comparing responses obtained in the LSD tolerant dog to the appropriate non-tolerant control. Superscripts indicating levels of significance for these comparisons are located above the S.E.

	Saline		LSD 10 μg/kg		AMPH 3.2 mg/kg		PEA 9.6 mg/kg	
	Non-tolerant dog	LSD tolerant dog	Non-tolerant dog	LSD tolerant dog	Non-tolerant dog	LSD tolerant dog	Non-tolerant dog	LSD tolerant dog
Flexor reflex	−276	−356	$^{0.05}$2591	−44	$^{0.01}$1852	1202	$^{0.01}$823	1005
(% · min)	±144	±153	±960	±131$^{0.05}$	±377	±217	±75	±120
Stepping reflex	No stepping	No stepping	$^{0.01}$32	No	37	39	$^{0.01}$7	11
(min to onset)			±3	stepping$^{0.01}$	±3	±1	±1	±1$^{0.01}$
Respiration	8	−14	$^{0.05}$3122	598	$^{0.05}$2258	3130	$^{0.01}$3523	2701
(breaths/min · min)	±132	±109	±1165	±127$^{0.05}$	±852	±1085$^{0.05}$	±640	±922
Pulse	2	108	$^{0.05}$392	−122	$^{0.01}$−825	−763	56	−182
(beats/min · min)	±63	±101	±117	±73$^{0.05}$	±179	±203	±145	±35$^{0.01}$
Pupillary diameter	−11.6	−3.4	$^{0.01}$21.8	3.5	$^{0.01}$157.3	155.8	38.2	30.2
(mm · min)	±6.0	±5.3	±5.2	±3.7$^{0.05}$	±15.2	±12.7	±4.8	±4.6
Nictitating membrane	1.2	8.9	−6.5	3.6	$^{0.01}$−45.6	−30.8	$^{0.01}$−8.7	−5.6
(mm · min)	±4.7	±4.3	±2.1	±5.0	±11.6	±8.9	±1.7	±2.3$^{0.05}$
Temperature	−0.2	0.2	$^{0.05}$0.1	0.2	$^{0.01}$0.9	1.2	$^{0.01}$0.6	0.5
(°C change)	±0.0	±0.4	±0.1	±0.3	±0.2	±0.2	±0.1	±0.3
Skin twitch	1.8	−13.6	−1.7	−13.1	$^{0.01}$65.0	36.9	$^{0.05}$28.9	23.8
(sec · min)	±6.7	±15.0	±24.5	±19.6	±15.9	±18.9	±12.2	±9.0
n	6	6	6	6	6	6	6	6

3.3. β-Phenethylamine (PEA)

PEA (9.6 mg/kg/12 min) markedly facilitated the flexor reflex, elicited the stepping reflex, increased respiration, caused a large mydriasis, retracted the nictitating membrane, produced analgesia as shown by the increased skin twitch reflex latency, and elevated body temperature (fig. 2). Although only results of the first PEA infusion are shown in table 1, these actions were significant for all three infusions. In fig. 2 it can be seen that prior to the second infusion there were slight residual effects of the first PEA infusion upon the flexor reflex amplitude, respiratory rate and pupillary diameter. The trend for pulse rate to decrease during each infusion was not significant in the pretolerant state. In the nontolerant dog the only evidence for tachyphylaxis was observed on the flexor reflex for which the facilitation produced by the third infusion was significantly less than that of the first ($P < 0.05$). However, the degree of tachyphylaxis was not prominent. Fig. 3 shows that PEA made the dogs restless, and this was accompanied by darting eye movements, head tossing and head rocking. When PEA was infused into LSD tolerant dogs there was, with two exceptions, a general lack of cross tolerance to both the physiologic and behavioral changes (figs. 2 and 3). Cross tolerance for the stepping reflex was evidenced by a slower onset of stepping for the first two infusions and only for the first infusion did the nictitating membrane retraction show cross tolerance. In contrast to the pretolerance study, pulse rate was consistently and significantly slower during all three PEA infusions.

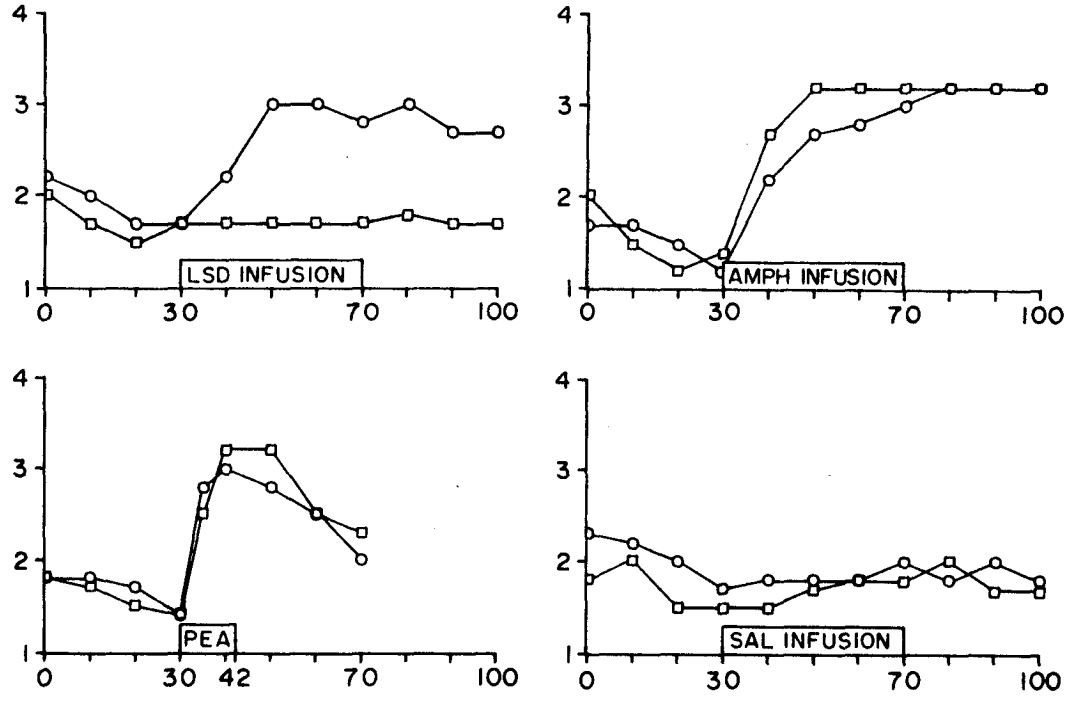

Fig. 3. Demonstration of direct tolerance to the behavioral effects of LSD (10 μg/kg) and the absence of cross tolerance to d-amphetamine (3.2 mg/kg) and β-phenethylamine (9.6 mg/kg) in chronic spinal dogs tolerant to 30 μg/kg/day of LSD. There were 6 dogs in each group. o———o, Pretolerant controls; □———□, LSD tolerance. Ordinates: 1, sleeping; 2, quiet; 3, restless; 4, struggling. Abscissae: time (min).

3.4. Saline

Saline control experiments demonstrated no physiologic (Vaupel et al., 1977) or behavioral (Nozaki et al., 1977) changes.

4. Discussion

Our observations on the somatomotor, autonomic and behavioral effects of PEA replicated many of the findings of Martin and Eades (1974). Generally, the pharmacologic effects of PEA resembled the actions of amphetamine more than LSD. In this respect our findings agree with the similarities between amphetamine and PEA reported on certain types of locomotor activity, appetite suppression, temperature (Mantegazza and Riva, 1963), EEG activation, visual evoked responses and behavioral aggression and arousal (Sabelli et al., 1975). However, the spinal cord reflex activity of PEA differs from amphetamine particularly with respect to the degree of facilitation and the evocation of the stepping reflex and appears to be like LSD in these respects. But consideration of the absence of cross tolerance to the facilitative effect and the relatively incomplete cross tolerance to stepping suggest a mode of action different from LSD.

Previous studies employing selective antagonists that interact with monoaminergic systems have attempted to pharmacologically differentiate the mechanisms mediating the spinal cord actions of LSD and amphetamine in the chronic spinal dog. Both the facilitation and stepping produced by LSD and tryptamine are antagonized by cyproheptadine but not by phenoxybenzamine. This distinguishes LSD and tryptamine from 5-hydroxytryptophan whose spinal cord effects are not antago-

nized by cyproheptadine (Martin and Eades, 1970). For this reason LSD is thought to act upon tryptaminergic instead of serotonergic receptors. A noradrenergic mechanism appears to be at least partly responsible for the flexor reflex facilitation produced by amphetamine since phenoxybenzamine and not cyproheptadine (Martin and Eades, 1967, 1970) or pimozide (Vaupel, unpublished data) antagonize this effect. The spinal cord interactions of PEA with antagonists are currently being investigated.

Not only was there a complete lack of cross tolerance to amphetamine in the LSD tolerant dog which corroborates the clinical report of Rosenberg et al. (1963) but there were instances in which the actions of amphetamine were enhanced. First, two LSD tolerant dogs developed larger and more prolonged hyperthermic effects than in the pretolerant condition although these effects are not reflected in the statistics (table 1; fig. 1). In one instance this led to the death of a dog, in the second case, ice and pentobarbital were used to lower body temperature. As previously described, cross tolerance studies using 2.0 mg/kg of amphetamine were repeated specifically to avoid marked hyperthermia; however, in these experiments the restless behavior and pupillary effects of amphetamine were significantly potentiated. Trends for comparable enhanced behavioral activity in LSD tolerant dogs were observed for amphetamine (3.2 mg/kg) and PEA (fig. 3) although the effects were not significant.

In summary, we have shown that PEA produces several amphetamine-like actions, and LSD tolerant animals which exhibit no cross tolerance to amphetamine show little cross tolerance to PEA. Once tolerance to LSD was established, evidence was obtained that the dogs may be hyperresponsive to amphetamine.

References

Durden, D.A., S.R. Philips and A.A. Boulton, 1973, Identification and distribution of β-phenylethylamine in the rat, Can. J. Biochem. 51, 995.

Inwang, E.E., A.D. Mosnaim and H.C. Sabelli, 1973, Isolation and characterization of phenylethylamine and phenylethanolamine from human brain, J. Neurochem. 20, 1469.

Mantegazza, P. and M. Riva, 1963, Amphetamine-like activity of β-phenethylamine after a monoamine oxidase inhibitor, J. Pharm. Pharmacol. 15, 472.

Martin, W.R. and C.G. Eades, 1967, Pharmacologic studies of spinal cord adrenergic and cholinergic mechanisms and their relation to physical dependence on morphine, Psychopharmacologia 11, 195.

Martin, W.R. and C.G. Eades, 1970, The action of tryptamine on the dog spinal cord and its relationship to the agonistic actions of LSD-like psychotogens, Psychopharmacologia 17, 242.

Martin, W.R. and C.G. Eades, 1974, Effects of phenethylamine (PEA) in the chronic spinal dog, Pharmacologist 16, 205.

Martin, W.R., C.A. Haertzen and B.B. Hewett, 1978, Psychopathology and pathophysiology of narcotic addicts, alcoholics and drug abusers, in: Psychopharmacology: A Generation of Progress, eds. M.A. Lipton, A. Dimascio and K.F. Killam (Raven Press, New York) p. 1591.

Nozaki, M., D.B. Vaupel and W.R. Martin, 1977, A pharmacologic comparison of 3,4-methylenedioxyamphetamine and LSD in the chronic spinal dog, European J. Pharmacol. 46, 339.

Risner, M.E. and B.E. Jones, 1977, Characteristics of β-phenethylamine self-administration by dog, Pharmacol. Biochem. Behav. 6, 689.

Rosenberg, D.E., A.B. Wolbach, E.J. Miner and H. Isbell, 1963, Observations on direct and cross tolerance with LSD and d-amphetamine in man, Psychopharmacologia 5, 1.

Saavedra, J.M., 1974, Enzymatic isotopic assay for and presence of β-phenethylamine in brain, J. Neurochem. 22, 211.

Sabelli, H.C., A.D. Mosnaim and A.J. Vazquez, 1974, Phenylethylamine: possible role in depression and antidepressive drug action, in: Neurochemical Coding of Brain Function, eds. R.D. Myers and R.R. Dracker-Colin (Plenum Publishing Corporation, New York) p. 331.

Sabelli, H.C., A.J. Vazquez and D. Flavin, 1975, Behavioral and electrophysiological effects of phenylethanolamine and 2-phenylethylamine, Psychopharmacologia 42, 117.

Vaupel, D.B., M. Nozaki and W.R. Martin, 1977, A pharmacologic comparison of 2,5-dimethoxyamphetamine and LSD in the chronic spinal dog, Drug Alc. Dependence 2, 45.

Willner, J., H.F. LeFevre and E. Costa, 1974, Assay by multiple ion detection of phenylethylamine and phenylethanolamine in rat brain, J. Neurochem. 23, 857.