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Lysergic Acid Diethylamide Antagonizes Shaking Induced in Rats by Five Chemically Different Compounds

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Abstract. Thyrotropin-releasing hormone (TRH), sodium valproate, AG-3-5 (1-[2-hydroxyphenyl]-4-[3-nitrophenyl]-1,2,3,6-tetrahydropyrimidine-2-one), RX 336-M (7,8-dihydro-5′,6′-dimethylcyclohex-5′-eno-1′,2′,8′,14 codeinone), and Sgd 8473 (α-[(4-chlorobenzylideneamino)-oxy]-isobutyric acid) each induced repetitive shaking of the body of rats after intraperitoneal injection. This action of the five diverse chemicals appears to be subserved by a common pharmacological component, because pretreatment with *d*-lysergic acid diethylamide (0.03 – 1.0 mg kg^{-1}, s.c.) attenuated the shaking behavior in a dose-related manner, and cross tolerance was found between RX 336-M and TRH, sodium valproate, and AG-3-5.

Key words: LSD – 'Wet-dog' shakes – Tolerance – Cross tolerance

Repetitive shaking of the body ('wet-dog' shake behavior) (Wikler et al., 1960) is commonly observed in the rat during precipitated abstinence (Bläsig et al., 1973) or after abrupt withdrawal (Martin et al., 1963) of narcotic analgesics. Body shakes are also elicited in normal rats after acute parenteral injection of the experimental compounds AG-3-5 (Burford and Chappel, 1972) and Sgd 8473 (Jahn and Mixich, 1976), and in pentobarbital-anaesthetized rats after intracisternal injection of thyrotropin-releasing hormone (TRH) (Prange et al., 1974). An interest in the nature of this behavior has been greatly stimulated by the work of Wei and his colleagues (Wei et al., 1975, 1976; Wei, 1976), Francis et al. (1975), and recently by the observation that endogenous morphinomimetic brain peptides can induce shaking movements in drug-naive rats after intraventricular injection (Bloom et al., 1976; Gispen et al., 1976; Leybin et al., 1976). In the present paper, we report that 'wet-dog' shakes also occur in rats after parenteral injection of two more chemically unrelated compounds – the antiepileptic drug, sodium valproate (Lance and Anthony, 1977; Pinder et al., 1977) and a dihydrocodeinone with weak antinociceptive and weak morphine antagonistic properties, RX 336-M (Cowan and Macfarlane, 1976). These two compounds, along with TRH, AG-3-5, and Sgd 8473, seem to act on pharmacologically similar pathways since, in each case, shaking episodes are greatly reduced if the rats are pretreated with *d*-lysergic acid diethylamide (LSD). Further evidence of a common mechanism underlying the shaking behavior is suggested by our finding that cross tolerance exists between RX 336-M on the one hand and TRH, AG-3-5, and sodium valproate on the other.

MATERIALS AND METHODS

Animals. The experiments were carried out on male Sprague Dawley albino rats (Olac) weighing 120 – 140 g.

Compounds. Sodium valproate (sodium *n*-dipropylacetate, Reckitt & Colman), LSD tartrate (Spofa, Prague), and TRH were dissolved in 0.9% w/v NaCl solution (saline); Sgd 8473 (α-[(4-chlorobenzylideneamino)-oxy]-isobutyric acid) was dissolved in saline and brought to pH 4 with $NaHCO_3$ solution. AG-3-5 (1-[2-hydroxyphenyl]-4-[3-nitrophenyl]-1,2,3,6-tetrahydropyrimidine-2-one) and RX 336-M maleate (7,8-dihydro-5′,6′-dimethylcyclohex-5′-eno-1′,2′,8′,14 codeinone) were suspended in saline with 0.5% Tween 80. With sodium valproate, LSD, and RX 336-M, the doses quoted refer to the particular salt.

Procedure. Groups of four rats were placed in individual Plexiglas (ventilated) boxes (22 × 11 × 11 cm) for 15 min between 9:00 and 11:00 h. The oesophageal temperature of each rat was then recorded using an oral thermistor probe and electric thermometer (Light laboratories, Brighton, England). The rats were injected s.c. with either saline (5 ml kg^{-1}) or LSD (0.03 – 1.0 mg kg^{-1}) and observed. At 15 min later, temperatures were again recorded, the test compound

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or saline (10 ml kg^{-1}) was injected i.p., and the number of 'wet-dog' shakes during the following 30 min was noted. Final temperatures were taken at the end of this period. Each treatment was repeated with additional groups of four rats between 13:00 and 16:00 h on the same day.

In the study of tolerance and cross tolerance, five groups of eight rats (80 – 90 g initially) were individually housed and injected i.p. with RX 336-M (6 mg kg^{-1}) at 8:30 h and 16:30 h for seven consecutive days. One group was observed after the first injection so that tolerance to the shaking and hyperthermic effects of RX 336-M could subsequently be assessed. The final injections were staggered so that successive groups of rats were injected every 30 min. At 16 h later, each group was challenged i.p. with approximately equipotent (shaking) doses of one of three drugs (RX 336-M, AG-3-5, or Sgd 8473) or optimal doses of one of two drugs (TRH and sodium valproate). The rats were then observed for 30 min.

Group means for 'wet-dog' shakes were compared using the Mann-Whitney *U*-test; temperature comparisons were made using Student's *t*-test for paired data. All significance levels are two-tailed.

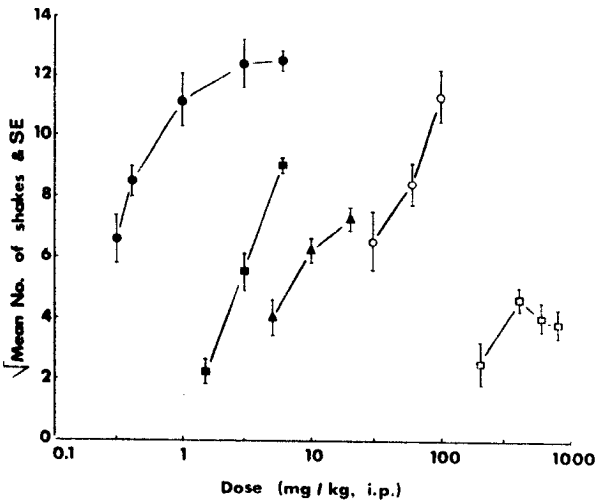

Fig. 1. Relationship between dose of test compound (on a log scale) and incidence of 'wet-dog' shakes. *Ordinate:* square root of total number of shakes occurring during 30 min after injection to groups of eight individually-housed rats. *Key:* (●) AG-3-5, (■) RX 336-M, (▲) TRH, (○) Sgd 8473, and (□) sodium valproate

RESULTS

At 3 – 5 min after injection of each test compound, rats displayed extensive grooming of the face and flanks – a behavior also induced by certain peptides derived from lipotropin (Gispen et al., 1976) and ACTH (Gispen et al., 1975). Spontaneous body shakes, involving a rapid twisting of the entire trunk and with forepaws leaving the ground, were first observed 3 – 8 min after each dose of test compound (Fig. 1). Ptosis and diarrhea developed over the 30-min session. Rats injected with sodium valproate did not defecate excessively but became increasingly sedated as the dose increased. Rats injected with AG-3-5 showed forepaw tremor, writhing, and escape attempts, and they frequently gnawed the floor. Statistically significant temperature changes (mean rises of 1.0 – 1.3° C, $P < 0.01$) only occurred after the two higher doses of AG-3-5, RX 336-M, and Sgd 8473.

LSD (1 mg kg^{-1}) produced a syndrome in all rats, particularly during the 15 min after injection, consisting of aimless sniffing, ear twitching, side-to-side head movements, defecation, and a reduced pelvic elevation (see also Dixon, 1968). Only one whole body 'wet-dog' shake was observed. The excessive grooming and shaking behavior induced by each test compound was attenuated by LSD in a dose-related manner (Table 1). At the top dose of 1 mg kg^{-1}, LSD essentially abolished 'wet-dog' shakes in rats receiving RX 336-M, TRH, and sodium valproate. The usual hyperthermic response of rats to RX 336-M and Sgd 8473 did not occur in the presence of 1 mg kg^{-1} of LSD; it should be stressed, however, that this dose of LSD, by itself, caused significant hypothermia (0.50 ± 0.12° C, SEM, $P < 0.01$) during the period 15 – 45 min after injection.

In the study of tolerance and cross tolerance, the incidence of shaking over each 30-min observation period was compared with results previously obtained

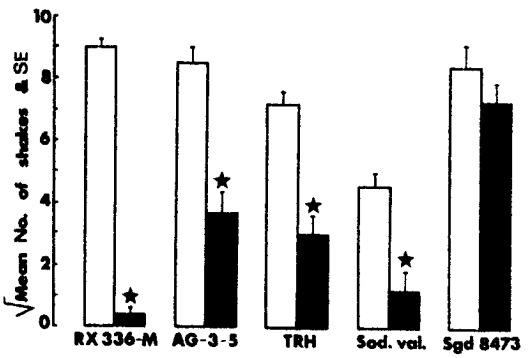

Fig. 2. Extent of cross tolerance between RX 336-M and four other agonists that induce shaking in rats. *Clear histograms:* square root of total number of shakes occurring during 30 min after acute i.p. administration to groups of eight drug-naive rats of RX 336-M (6 mg kg^{-1}), AG-3-5 (0.40 mg kg^{-1}), TRH (20 mg kg^{-1}), sodium valproate (400 mg kg^{-1}), or Sgd 8473 (60 mg kg^{-1}). RX 336-M group and further four groups of eight rats then received 14 injections of RX 336-M (6 mg kg^{-1}, i.p.) twice daily for 7 days. *Solid histograms:* incidence of shaking after administration of same doses of test compound on day 8. * $P < 0.001$, Mann Whitney *U*-test

in drug-naive animals (Fig. 2). With RX 336-M, tolerance developed to the excessive shaking and grooming, but not to the diarrhea and hyperthermia. Rats rendered tolerant to RX 336-M displayed significantly fewer ($P < 0.001$) shaking episodes than drug-naive animals when challenged with AG-3-5, TRH, or sodium valproate. In contrast, the number of 'wet-dog' shakes induced by Sgd 8473 was reduced, but not significantly, in rats pretreated with RX 336-M. At 72 h after the Sgd 8473 challenge, four rats from this latter group received a final injection of RX 336-M

Table 1. Effect of LSD on chemically induced shaking

Compound	Dose mg kg^{-1}	Mean No. of shakes ± SEM after pretreatment with LSD				
		0	0.03	0.1	0.3	1.0 mg kg^{-1}
AG-3-5	0.4	73.5 ± 8.9		35.3 ± 9.8*		5.4 ± 2.9***
RX 336-M	6	112.2 ± 8.8	93.3 ± 7.6**	63.2 ± 5.2***		
	6	86.3 ± 9.6				0.8 ± 0.8***
TRH	20	53.4 ± 6.7		8.0 ± 2.2***		0***
Sgd 8473	60	74.4 ± 9.4		60.6 ± 5.0		8.3 ± 2.7***
Sodium valproate	400	22.6 ± 3.7		17.5 ± 3.0	5.3 ± 1.6**	0.5 ± 0.3***

Groups of 8 rats were pretreated s.c. with LSD or saline; 15 min later a standard dose of test compound was injected s.c. and the number of 'wet-dog' shakes occurring during the next 30 min was noted. Statistical significance (Mann Whitney U-test): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

(6 mg kg^{-1}). Extended tolerance to RX 336-M was suggested since no shaking occurred.

DISCUSSION

The shake-inducing properties of TRH, sodium valproate, AG-3-5, RX 336-M, and Sgd 8473 have been compared in rats. Since test doses of TRH and sodium valproate elicited shaking but caused no significant temperature changes, it is unlikely that the shaking induced by AG-3-5, RX 336-M, and Sgd 8473 was directly related to the associated hyperthermia; indeed, shaking was virtually absent at the end of the tolerance study with RX 336-M, although marked rises in temperature still persisted. Parenthetically, it is of interest that shaking was linked with both hyperthermia and unchanged temperatures in the present study, whereas this behavior is usually associated with hypothermia in morphine-abstinent rats (e.g., Ary et al., 1977).

When the animals were pretreated with *d*-lysergic acid diethylamide (0.03 – 1.0 mg kg^{-1}), the shaking behavior was antagonized in a dose-related manner. This suggests that the shake-inducing action of the five diverse chemicals is subserved by a common pharmacological component.

What properties of LSD are responsible for the attenuating effects? Based on neurochemical (Andén et al., 1968), neurophysiological (Aghajanian, 1976), and behavioral (Trulson et al., 1976) evidence, LSD has been considered to act, at least in part, as an agonist at certain central serotonin receptors. Additionally, LSD may directly stimulate dopamine receptors in the rat CNS (Kelly and Iversen, 1975; Pieri et al., 1975; Nichols, 1976). These properties are mentioned in particular since they are shared by nomifensine (8-amino-1,2,3,4-tetrahydro-2-methyl-4-phenylisoquinoline) (Costall et al., 1975; Mogilnicka et al., 1976) and quipazine (2-[1-piperazinyl]quinoline) (Costall and Naylor, 1975; Jacoby et al., 1976), two compounds that also antagonize the shaking induced by a standard dose (6 mg kg^{-1}) of RX 336-M (unpublished observations). As yet, it is not known whether optimal antagonism of shaking is associated with stimulation of serotonin receptors, dopamine receptors, or both.

Such considerations, of course, are based on only a small number of possible antagonists and it is likely that shaking behavior induced by each of the five test compounds will be affected by other pharmacological agents. Indeed, morphine suppresses the frequency of 'wet-dog' shakes induced by Sgd 8473 (Jahn and Mixich, 1976) and AG-3-5 (Wei, 1976), while the behavioral effects of RX 336-M, TRH, and sodium valproate are likewise attenuated by morphine but not by the narcotic antagonist naloxone (unpublished observations). In the past, much has been learned about those compounds or classes of compound that affect simple behavioral baselines in animals. It remains to be seen if congeners of LSD will be characterized in the shaking-model as, say, serotoninergic agonists, in relation to data from seemingly more specific tests (Andén et al., 1968; Aghajanian, 1976), or, again, if the shaking behavior is differentially sensitive to analgesics acting on a particular opiate receptor (Martin et al., 1976a, b).

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