Reports

Patterns of Tolerance to Lysergic Acid Diethylamide and Mescaline in Rats

D-Lysergic acid diethylamide (LSD-25) and mescaline are chemically distinct agents which induce similar alterations of psychological and autonomic function in man. Nonpsychotic human beings clearly develop tolerance to the behavioral and most of the autonomic effects of LSD-25 (*1, 2*), but mescaline has not been thoroughly studied in these respects. To our knowledge, the details of tolerance to the behavioral effects of LSD-25 and mescaline in rats have not been reported. Future investigation of neurochemical mechanisms underlying both tolerance and the action of psychosomimetic agents would be facilitated if patterns of tolerance could be delineated for two such agents in a single animal system. On the basis of findings discussed here (*3*), we report tolerance to the behavioral impairments due to LSD-25 and mescaline in the rat and the absence of tolerance to LSD-25-induced bradycardia.

In single-dose experiments, Winter and Flataker (*4*) found that LSD-25 impaired the performance of rats that had been trained to climb a rope; climbing time increased as a linear function of the logarithm of the dose. The method is not suitable for refined analysis of behavioral mechanisms, but it does quantitatively reflect the effects of dose on the complex of perceptual, motor, and coordinative functions necessary for efficient climbing. We thought that any increase in climbing time due to the drug would gradually disappear if tolerance to the drug were induced by daily injections. The rat, placed in the arena, is taught to grasp and climb a rope which extends 160 cm to a platform. We used neither electric shock nor noise as reinforcement but always gave food for the rat to eat or hoard at the end of a climb. Trained for 19 days, with prior food deprivation for the first 5 days, the rat establishes a stable climbing time, usually after 6 or 7 days. It maintains this stable time for months thereafter, often without specific intervening practice; these stable control times are not affected by placebo injection or satiety. Using male Sherman rats, 180 to 200 g in weight, we encountered occasional aversive reactions which seemed contingent specifically upon repeated intraperitonal drug injections; therefore, all trained animals received three intraperitoneal placebo injections at 48-hour intervals prior to initial drug injections, and during each injection they were fed a pellet. With each drug test group (A, B, and C) there was a placebo group. Failure to begin to climb from the arena within 60 seconds was scored as complete impairment; tests were run at regular intervals after each injection (Fig. 1).

Seven rats (group A) that were given daily intraperitoneal injections of 130 μg of LSD-25 per kilogram showed virtually complete tolerance in 4 days (Fig. 1). It is important to note that the gross behavioral effects of the drug (for example, piloerection, nonresponsiveness to approach, confusion, and "spontaneous distractibility") correlate with scored results. Development of tolerance is rapid, appearing in some animals as early as 1 day after the initial injection and in all animals by 4 days; tolerance can be maintained thereafter by daily injection. Findings were similar when the daily dose was increased, over 4 days, from 130 to 260 μg/kg.

A second group (B) of six rats received intraperitoneal injections of 130 μg of LSD-25 per kilogram at 48-hour intervals over a period of 14 days and displayed variability in the development of tolerance. One rat failed to acquire any appreciable tolerance, three showed incomplete tolerance at the last injection, and two showed tolerance at the third injection, reflecting individual differences in the rate of formation and decay of tolerance. It appears that the 48-hour interval may be a critical limit for the processes involved, for we encountered no tolerance in 72-hour tests with these doses.

Six rats (group C) received daily injections of mescaline, 10 mg/kg, over 10 days. Gross behavioral effects and climbing impairment following a single dose are similar to the effects and impairment following the injection of LSD-25, but with mescaline the onset is later; impairment in climbing begins at 10 rather than 5 minutes after injection, with a peak at 20 rather than 10 minutes, and a return to normal at 50 to 60 rather than at 30 to 45 minutes. Nor is tolerance development as rapid; three of the rats were completely tolerant at the fourth injection, one at the fifth, and two at the seventh. Since tolerance develops with respect to the behavioral effects of both drugs, future research must not only inquire into a possible common biochem-

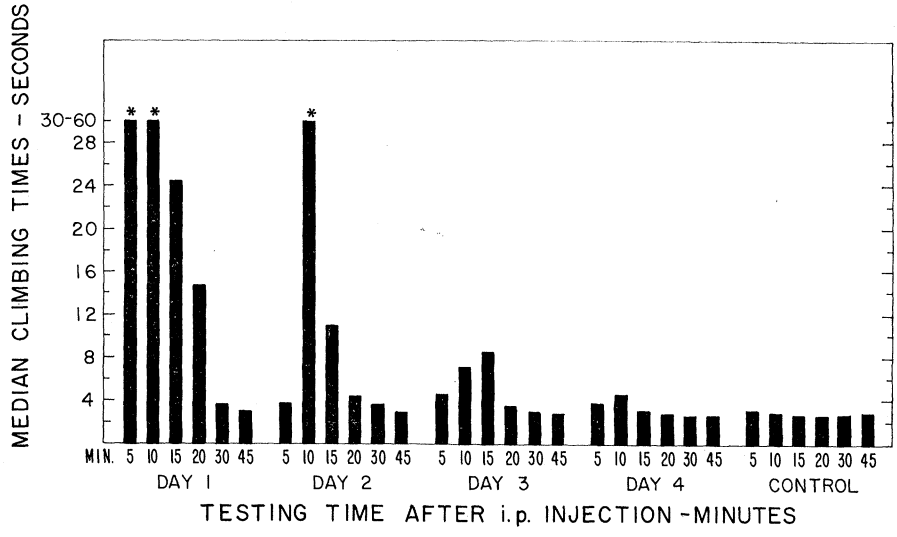

Fig. 1. Median climbing time for group A rats, showing development of tolerance with intraperitoneal injection of LSD-25 (130 μg/kg). Asterisk indicates failure to climb after 60 seconds in the arena.

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ical or neural mechanism but must also explain the relative difficulty with which tolerance to mescaline is established.

To distinguish drug tolerance from "learning," the loss of impairment must be contingent upon the pattern of drug injections and not upon climbing experience. When we injected rats daily with the drug but omitted the climbing test on the second and third injection days, climbing was no longer impaired on the fourth injecion day. Conversely, when we injected rats daily with placebos and permitted them to climb daily, climbing was impaired with drug injection on the fourth day. Finally, rats in groups A, B, and C were permitted to lose tolerance; climbing was impaired with the first injection of the drug, and with daily injections the animals regained tolerance, each in its own characteristic pattern.

In a set of experiments on cardiac effects, we found that rats restrained in a holder displayed a tachycardia which was affected only slightly by single doses of LSD-25. In order to record bradycardia in rats, subcutaneous needle electrodes were attached to the limbs, allowing the animal freedom of movement during electrocardiographic recordings. Intraperitoneal placebo injections caused a tachycardia under these conditions, and injections of LSD-25 induced bradycardia, the pulse decreasing from a base of 415 to 300 per minute. Daily intraperitoneal injections of at least 175 μg of LSD-25 per kilogram were administered for as long as 12 days. Bradycardia was most marked within the first 30 minutes following injection and began to decrease at 90 minutes. Although the degree of bradycardia varied from day to day, we found no clear indications of the development of tolerance. Similarly, chronic experiments with mescaline-induced bradycardia in rats have failed to demonstrate tolerance (*5*). The bradycardia induced with LSD-25 has been thought to be due to a central mechanism, since, in the cat, LSD-25–induced bradycardia is abolished by spinal section (*6*). The peripheral anticholinesterase effects of LSD-25 would be minimal in the dosage range employed here (*2*).

Our findings and those of others suggest a pattern underlying the development of tolerance to the effects of LSD-25. No tolerance is manifest with respect to bradycardia and the respiratory arrest that occurs with high dosages (*7*); these two effects probably involve centers in the caudal brain stem. Pyrexia, mydriasis, and piloerection are autonomic effects of LSD-25 to which tolerance has been shown to develop (*6, 8*); more rostral brain-stem mechanisms have been implicated in the origin of these responses (*6*). Similarly, rostral mechanisms may be involved both in the behavioral effects and in the tolerance observed with respect to both psychosomimetic drugs. The rostral mechanisms which are involved in electroencephalographic and behavioral arousal and which show "habituation" to sensory stimulation (*9*) could as well show tolerance to chemical stimulation. This suggests that both neurochemical and electroencephalographic studies would be useful in investigating the basis of tolerance. Since tolerance may be a phenomenon characteristic of the entire group of psychosomimetic drugs, comparative studies of autonomic behavioral and electroencephalographic effects should be attempted.

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References and Notes

1. H. Isbell *et al.*, *A.M.A. Arch. Neurol. Psychiat.* 76, 468 (1956).
2. H. A. Abramson, Ed., *Neuropharmacology* (Josiah Macy, Jr. Foundation, New York, 1956), pp. 29, 235, 295.
3. This study was supported by U.S. Public Health Service grant No. M1204R.
4. C. L. Winter and L. Flataker, *Proc. Soc. Exptl. Biol. Med.* 92, 285 (1956).
5. L. B. Speck, *J. Pharmacol. Exptl. Therap.* 9, 569 (1957).
6. E. Rothlin and A. Cerletti, in *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry,* L. Cholden, Ed. (Grune & Stratton, New York, 1956).
7. E. Rothlin, *J. Pharm. and Pharmacol.* 9, 569 (1957).
8. J. H. Gogerty and J. M. Dille, *J. Pharmacol. Exptl. Therap.* 116, 450 (1956).
9. S. Sharpless and H. Jasper, *Brain* 79, 655 (1956).

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Antimicrobial Activity of Horny Corals

Recent studies (*1*) on the occurrence of antibiotic substances in marine organisms have revealed some interesting antimicrobial properties of gorgonian corals, belonging to the phylum Coelenterata. Corals were collected for this work from reefs located off the southern coast of Puerto Rico. For the assays of antibacterial activity in the various materials, many indicating marine bacteria were isolated from the same region and grown in Difco nutrient agar made with sea water. Other common test microorganisms were grown in ordinary nutrient agar made with distilled water. Small pieces of coral, or various extracts from different species of coral, were placed on nutrient agar plates, which had been inoculated with the appropriate indicating microbes. After incubation for about 16 hours, zones of microbial inhibition became conspicuous around the fragments of coral and paper discs containing extracts from active corals.

Among the corals which showed antibacterial action were the following species: *Antillogorgia turgida, A. americana, Rhipidogorgia flabellum, Briareum asbestinum, Plexaura homomalla, Plexaurella dichotoma,* and *Plexauropsis crassa.* The sea whip, *Antillogorgia turgida,* was especially striking in its action against numerous marine bacteria, *Clostridium feseri, Micrococcus aureus, Bacillus subtilis,* and *Escherichia coli.* Strains of penicillin-resistant *Micrococcus* were equally susceptible to inhibition by extracts from *Antillogorgia.* Unsusceptible organisms included *Lactobacillus casei, Candida albicans, Kloeckera brevis, Cryptococcus neoformans,* and *Saccharomyces cervisiae.* It was easily demonstrated that antimicrobial activity could be extracted from both fresh and dried materials of sea whips, sea fans, and plexaurid corals, by means of water or other common solvents. The active principle appears not to be located in the brown core of the horny corals, but it is present in the outer, gray-purple cortex. This suggests that the activity is probably not associated with halogenated gorgonin of the horny axis. Segments of the fine branches, as well as the large basal stems, showed very sharp zones of inhibition on agar plates containing marine bacteria. In contrast to these results with gorgonian corals, little or no antimicrobial activity could be detected in the species of stony corals that were tested. Examples of inactive species are *Acropora palmata, Porites porites, Millepora alcicornis,* and *Montastrea sp.*

It is not known whether the coral polyps or their associated zooxanthellae produce antibacterial substances. It is of interest to note that another large group of terrestrial symbionts, the lichens, commonly produce antibiotic substances (*2*). The increasing number of examples of naturally occurring chemical antagonism among numerous kinds of organisms lends support to the idea, expressed so well by Brian (*3*), that these phenomena are "not incompatible with the view that the capacity to produce antibiotics is a character conducive to fitness." Perhaps successful symbiosis may be enhanced by the antibiotic properties of the complex organization of fungi and algae in lichens or of animals and algae in gorgonian corals.

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