Pharmacology Biochemistry & Behavior, Vol. 21, pp. 333–337, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Naloxone Alters the Effects of LSD, DOM and Quipazine on Operant Behavior of Rats

DAVID J. MOKLER,[1] RANDALL L. COMMISSARIS,[2]
JUDITH W. HENCK AND RICHARD H. RECH[3]

Department of Pharmacology and Toxicology, Neuroscience Program
Michigan State University, East Lansing, MI 48824

Received 16 January 1984

MOKLER, D. J., R. L. COMMISSARIS, J. W. HENCK AND R. H. RECH. *Naloxone alters the effects of LSD, DOM and quipazine on operant behavior of rats.* PHARMACOL BIOCHEM BEHAV 21(3) 333–337, 1984.—Administration of the indolealkylamine hallucinogen d-lysergic acid diethylamide (LSD), the phenethylamine hallucinogen 2,5-dimethoxy-4-methylamphetamine (DOM) and the putative 5-hydroxytryptamine (5-HT) agonist quipazine all produced a dose-dependent decrease in fixed ratio (FR-40) response rates and a concomitant increase in the number of 10-second pause intervals. Although naloxone (4.0 mg/kg) had no effect on FR-40 responding *per se*, the pause-producing effects of LSD and, to a lesser extent, DOM were potentiated by pretreatment with naloxone. The action of quipazine on reinforcers was unaffected by combination with naloxone, while the effect on pause intervals was slightly attenuated by naloxone pretreatment. These data and previous studies suggest that the pause-producing effects of indolealkylamine and phenethylamine hallucinogens reflect their activation of a selective portion of brain 5-HT receptors. The potentiation of these effects by naloxone may relate to a modulation of central 5-HT systems by endogenous opioid mechanisms tending to restore an imbalance in various 5-HT pathways caused by the hallucinogenic 5-HT agonists. The more generalized disruptive effects of quipazine on brain 5-HT systems may be less susceptible to the endogenous opioid modulation or may actually combine with it to induce a greater disruption.

Naloxone LSD DOM Hallucinogens Quipazine 5-Hydroxytryptamine

FIXED ratio (FR) schedules of operant responding have served in the study of neurotransmitter mechanism(s) of hallucinogenic drug action in rats. Early studies reported that the administration of hallucinogenic agents to rats performing on a FR schedule resulted in a cessation of responding for some portion of the operant session [1–4, 25]. Difficulties in quantifying this effect and similar patterns of disruption ("pausing") after administration of non-hallucinogenic psychoactive agents (*d*-amphetamine [2]) led investigators to search for alternative behavioral tests. However, with the addition of a pause interval counter into the FR-40 operant program, we have been able to quantify this pausing and differentiate the pause-producing effects of hallucinogens from the slowed and erratic intrasession response rates produced by non-hallucinogenic psychoactive agents such as *d*-amphetamine and phenobarbital [5, 9, 11]. With this refinement, experiments on the disruption of FR operant responding by hallucinogenic drugs have indicated that brain 5-hydroxytryptamine (5-HT) neurons and/or receptors are involved [1, 4, 6, 9–11, 16, 20].

The narcotic antagonist naloxone potentiates the pause-producing effects of the indolealkylamine hallucinogens LSD and N,N-dimethyltryptamine (DMT) [13, 22, 23] and the phenethylamine hallucinogen mescaline [7]. Ruffing and Domino [23] reported that naloxone or naltrexone (opiate antagonists) potentiated LSD- and DMT-induced disruption of operant behavior, while low doses of morphine and methadone attenuated the disruptive effects of LSD and DMT. A further suggestion of a link between hallucinogens and opioid systems derives from the hallucinogenic effects in man of cyclazocine, an opioid agonist-antagonist [19]. The pattern of disrupted operant behavior in rats caused by cyclazocine is similar to that induced by the hallucinogens. However, this disruption is antagonized by naloxone as well as by the 5HT antagonist metergoline [14]. These findings suggest a possible interaction between 5HT and opioid systems in the behavioral effects of hallucinogenic drugs.

The present study examined in more detail the interactions between naloxone and LSD or DOM. In addition, the disruptive effects of the non-hallucinogenic 5-HT agonist quipazine were examined alone and in naloxone-pretreated subjects.

METHOD

Subjects

Male Sprague-Dawley rats (Spartan Farms, Haslett, MI), weighing 300–350 g at the start of the experiment, were main-

[1]Present Address: Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Box 613, MCV Station, Richmond, VA 23298.

[2]Present Address: College of Pharmacy, Wayne State University and Allied Health Professions, Detroit, MI 48202.

[3]Requests for reprints should be addressed to R. H. Rech.

tained at 75–80% of their free-feeding weights and were housed individually in a windowed room with a natural light cycle and free access to tap water.

Behavioral Apparatus

Testing was conducted between 1400 and 1600 hr in one of four standard operant chambers (LVE No. 143-20-215) located in sound-attenuating boxes and equipped with food pellet dispensers. Each chamber contained a single lever which required a force of 10–15 g to activate. All experimental events were controlled by electromechanical programming circuits. Two parameters of operant responding were monitored: (1) the number of reinforcers obtained (a reflection of the average response rate) and (2) the period of non-responding, or "pausing," per daily session. A pause interval counter was incorporated into the programming as described previously [5–11]. Briefly, a 10-sec timer was started at the beginning of the session and each response by the animal before 10 sec reset the timer without registering a count. If 10 sec elapsed without a response being made, a "pause interval" was recorded on a counter and the timer reset automatically.

Behavioral Procedure

The subjects were trained to press a bar for food reinforcement (45 mg food pellets; BioServe Inc., Frenchtown, NJ) on a FR-40 schedule, i.e., 40 presses to obtain one food pellet during daily 40 min sessions. Each animal was run in the same cage at the same time of day, six days a week, and then given supplementary food daily to maintain body weights. The effects of various doses of LSD (12.5–100 μg/kg) alone and combined with naloxone (4.0 mg/kg) were determined in one group of 8 rats. In another group of 8 rats the effects of DOM were examined at doses of 0.0312–2.0 mg/kg; in a separate group of 7 rats the combination of DOM and 4.0 mg/kg naloxone was examined. Similarly, the effects of quipazine (0.5–8.0 mg/kg) with or without pretreatment with 4.0 mg/kg naloxone were observed in two groups of 8 rats each. The order of doses was randomized for each rat. LSD, DOM and quipazine were administered immediately before the FR-40 session; naloxone was administered 5 min before the session.

Statistical Analysis

Drug effects were assessed by comparing data from the test day to the control day immediately prior to the test day. Dose-response relationships were compared using a two-way analysis of variance [17]. Individual points were compared using the least significant differences (LSD) test. In all statistical evaluations $p<0.05$ was used as the criterion for statistical significance.

Drugs

All drugs were dissolved in distilled water and administered intraperitoneally. LSD tartrate and DOM hydrochloride were obtained from the National Institute on Drug Abuse. Naloxone hydrochloride was purchased from Endo Laboratories (Garden City, NY) and quipazine maleate was purchased from Miles Laboratories (Elkhart, IN). Doses of LSD, DOM and naloxone refer to the weight of the salts; in order to facilitate comparison with earlier studies doses of quipazine maleate were converted to the weight of quipazine hydrochloride.

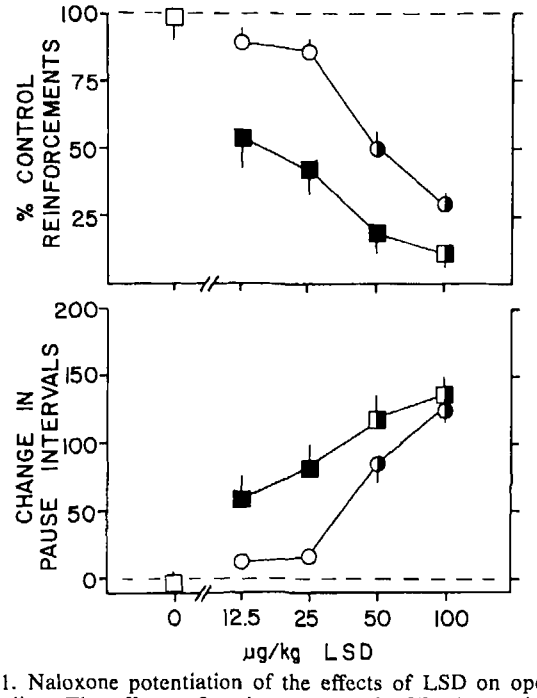

FIG. 1. Naloxone potentiation of the effects of LSD on operant responding. The effects of various doses of LSD alone (circles; 0-min pretreatment) and in combination with 4.0 mg/kg naloxone (squares; 5 min pretreatment) on % of control reinforcements (top panel) and change in number of pause intervals from control values (bottom panel) in FR-40 operant sessions are plotted. Each symbol and vertical bar represents the mean±S.E.M. obtained from 7 subjects. Shading of the right-half of the symbol indicates a significant difference from control values; shading of the left-half of the symbol indicates a significant difference from LSD alone ($p<0.05$, ANOVA, LSD test).

RESULTS

Control FR-40 operant responding was characterized by rapid response rates with brief (10–30 sec) pauses, usually following the delivery of the reinforcer, during daily sessions in well-trained subjects. Performance during control days consisted of 135±9 (mean±S.E.M., n=23) reinforcers earned and 27±4 pause intervals per session. The values for reinforcers received on individual control days before drug test days, expressed below as 100%, were invariably within the 95% confidence limits of the overall mean control value. Likewise, the number of pause intervals on individual control days prior to drug test days, expressed below as zero change in number of pauses, was within the 95% confidence limits of the overall control mean for number of pause intervals.

LSD causes a dose-related decrease in reinforcers earned along with a reciprocal increase in number of pause intervals (Fig. 1, circles), as has been described in previous studies [5, 9, 10, 20]. Naloxone (4 mg/kg) administered alone (open squares at 0 dose of LSD in Fig. 4) did not alter the percent of control reinforcers or the change in number of pause intervals from control values. This dose of naloxone combined with various doses of LSD (squares) potentiated the disruptive effects of the hallucinogen over the lower dose range for both the decrease in percent reinforcers, $F(1,62)=29.17$, and the increase in number of pause intervals, $F(1,62)=12.34$. However, the deficits with higher doses

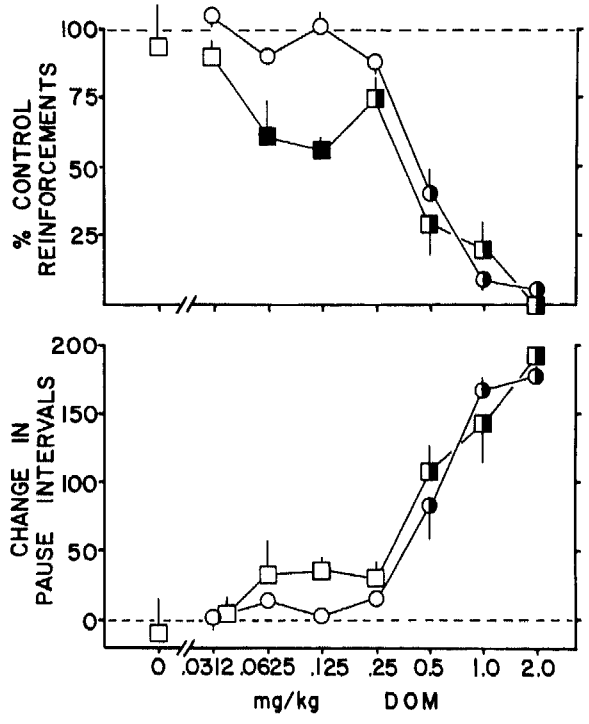

FIG. 2. Naloxone potentiation of the effects of DOM on operant responding. The effects of various doses of DOM alone (circles; 0 min pretreatment) and in combination with 4.0 mg/kg naloxone (squares; 5 min pretreatment) on FR-40 operant behavior (n=7) are plotted. See legend of Fig. 1 for further details.

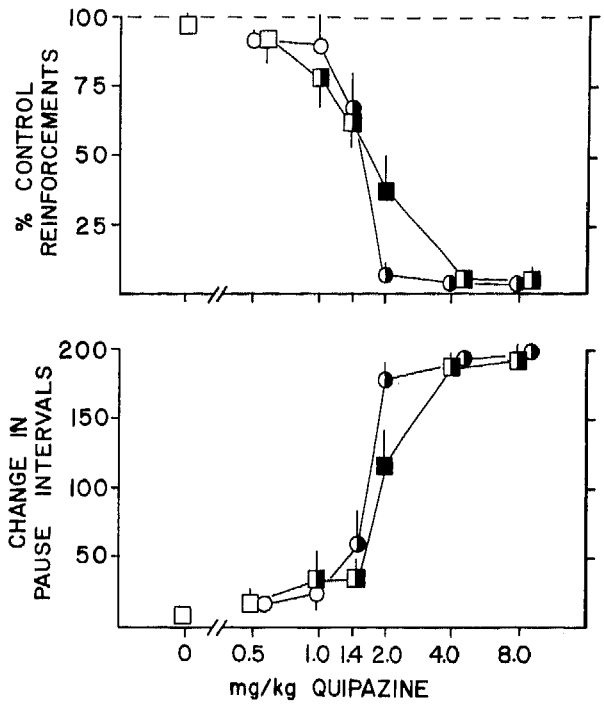

FIG. 3. Effects of naloxone on disruption by quipazine of operant responding. The effects of various doses of quipazine alone (circles; 0 min pretreatment) and in combination with 4.0 mg/kg naloxone (squares; 5 min pretreatment) are plotted (n=8). See legend of Fig. 1 for further details.

of LSD (100 μg/kg for reinforcers, 50 and 100 μg/kg for pauses) were not significantly potentiated by pretreating with naloxone. Therefore, the shifts in the dose-response curves are not parallel.

A decrease in reinforcers earned with a concomitant increase in pause intervals was also a dose-related characteristic of the effects of DOM (Fig. 2, circles), verifying previous findings [5, 6, 8, 9, 12]. Once again, naloxone administered alone failed to alter the reinforcement level or number of pauses from control values. Combining naloxone (squares) with lower doses of DOM, but not higher doses, resulted in a significant potentiation of the decrease in percent reinforcers, $F(1,96)=8.77$. Although individual values for the increase in number of pauses as a result of combining naloxone with DOM did not reach significance, the ANOVA for dose-response curves showed a slight potentiation, $F(1,96)=4.59$, $p<0.05$.

The effects of quipazine alone also resulted in a dose-related decrease in percent reinforcers and reciprocal increase in number of pause intervals (Fig. 3, circles), as noted in previous work [8, 12, 20]. However, pretreatment with naloxone did not potentiate the quipazine effect on reinforcers, $F(1,77)=1.58$, squares in Fig. 3. There was a single dose combination (4 mg/kg naloxone with 2 mg/kg quipazine) that actually caused a significant antagonism of the decrease in reinforcers brought about by 2 mg/kg of quipazine alone. Moreover, the increased number of pause intervals by 2 mg/kg quipacine alone was significantly reduced by pretreatment with naloxone. The ANOVA of dose-response curves for the pause effect also showed that the combination resulted in an antagonism of the increase in pause intervals as compared to quipazine alone, $F(1,77)=17.13$.

DISCUSSION

As reported previously, the hallucinogens LSD and DOM and the 5-HT agonist quipazine produced disruptions of FR-40 operant behavior characterized by "pausing" [5–12]. This contrasts with the effects of non-hallucinogenic psychoactive agents without 5-HT activity, which disrupt FR-40 responding in a pattern of slowed and erratic rates without any clear-cut "pausing" [5, 8, 9, 12]. The hallucinogens, quipazine and lisuride [20] produced significant increases in the number of pause intervals, while *d*-amphetamine, phenobarbital and chlorpromazine caused a considerably less dramatic increase. This "pausing" induced by the hallucinogens appears to relate to 5-HT agonistic actions.

Naloxone pretreatment potentiated the effects of LSD and, to a lesser extent, DOM, but not quipazine. These effects of naloxone presumably relate to an interference with endogenous opioid modulating systems, since this dose of naloxone was ineffective alone and appears to act as a "pure" antagonist [15]. The data with LSD are in agreement with Ruffing *et al.* [22,23], who studied a single dose of LSD. The effects of DOM extend our earlier studies with mescaline [7] showing a weak interaction between 4.0 mg/kg naloxone and low doses of mescaline and a prominent interaction between naloxone and larger doses of mescaline (i.e., a pattern opposite that of the interaction with LSD). This mescaline-type pattern was also seen in the interaction

of naloxone with DMT [22]. The naloxone-DOM combination, however, shifted dose-response curves similar to the interaction of naloxone with LSD, low doses of DOM being potentiated and higher doses being unaffected.

Since the naloxone potentiation of the hallucinogenic drugs is not manifested as parallel shifts in the dose-response curves, naloxone apparently does not act competitively on the same receptor system(s) affected by the hallucinogenic drugs. Therefore, this potentiation differs from that exerted by pretreating with 5,7-DHT, after which the dose-response curves for LSD and DOM effects on FR-40 were shifted to the left in a parallel fashion [10].

Antagonism of the effects of LSD and DMT on fixed-ratio responding by low doses of morphine and methadone [23] and by enkephalin analogs [24] further supports this proposed interaction with endogenous opioid systems. Cyclazocine-induced disruption of operant behavior is attenuated by naloxone or metergoline, and the antagonism is greater when the antagonists are combined [14]. This contrasts with the potentiation of hallucinogens by naloxone although the effects of LSD, DMT, DOM and mescaline are also antagonized by metergoline [9, 12, 20]. Metergoline antagonizes the disruption of operant behavior by quipazine [12,20] while naloxone protects slightly against this disruption. Furthermore, a subthreshold dose of cyclazocine does not alter the FR-40 effects of quipazine [21] while quipazine potentiates some of the effects of morphine [18]. Thus, although the hallucinogens and quipazine appear to disrupt FR-40 operant behavior by agonistic actions on brain 5-HT receptors [6, 9, 20], their mechanisms must differ in subtle ways as reflected by different interactions with naloxone and other agents. Previous studies have also suggested that LSD, DOM and mescaline exert their actions on central 5-HT systems by slightly different mechanisms and/or at different brain sites [9, 11, 20]. Additional research is obviously warranted to elaborate the precise differences in the ways in which opioid mechanisms interact with these various types of 5-HT agonists.

ACKNOWLEDGEMENTS

This study was supported by Grant No. DA-01836 from the National Institute on Drug Abuse. DJM, JWH, and RLC were supported by USPHS Training Grant GM-07392. The authors would like to thank Barbara Z. Steiber, Katharine W. Stoudt, and Sarah J. Robson for their excellent technical assistance.

REFERENCES

1. Appel, J. B. and D. X. Freedman. Chemically-induced alterations in the behavioral effects of LSD-25. *Biochem Pharmacol* **13**: 861–869, 1964.
2. Appel, J. B. and D. X. Freedman. The relative potencies of psychotomimetic drugs. *Life Sci* **4**: 2181–2186, 1965.
3. Appel, J. B. and D. X. Freedman. Tolerance and cross-tolerance among psychotomimetic drugs. *Psychopharmacologia* **13**: 267–274, 1968.
4. Appel, J., J. Joseph, L. Utsey, L. Hernandez and W. Boggan. Sensitivity to psychoactive drugs and the serotonergic neuronal system. *Commun Psychopharmacol* **1**: 541–551, 1977.
5. Commissaris, R. L., W. H. Lyness, J. J. Cordon, K. E. Moore and R. H. Rech. The effects of *d*-lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM) and *d*-amphetamine on operant responding in control and 6-hydroxydopamine-treated rats. *Pharmacol Biochem Behav* **13**: 621–626, 1980.
6. Commissaris, R. L., W. H. Lyness, K. E. Moore and R. H. Rech. Enhancement of the behavioral effects of 2,5-dimethoxy-4-methylamphetamine (DOM) by pretreatment with *p*-chlorophenylalanine. *Pharmacol Biochem Behav* **13**: 605–608, 1980.
7. Commissaris, R. L., K. E. Moore and R. H. Rech. Naloxone potentiates the disruptive effects of mescaline on operant responding in the rat. *Pharmacol Biochem Behav* **13**: 601–603, 1980.
8. Commissaris, R. L., D. R. Semeyn, K. E. Moore and R. H. Rech. The effects of 2,5-dimethoxy-4-methylamphetamine (DOM) on operant behavior: Interactions with other neuroactive agents. *Commun Psychopharmacol* **4**: 393–404, 1980.
9. Commissaris, R. L., W. H. Lyness, K. E. Moore and R. H. Rech. Differential antagonism by metergoline of the behavioral effects of indolealkylamine and phenethylamine hallucinogens in the rat. *J Pharmacol Exp Ther* **219**: 170–174, 1981.
10. Commissaris, R. L., W. H. Lyness, K. E. Moore and R. H. Rech. Central 5-hydroxytryptamine and the effects of hallucinogens and phenobarbital on operant responding in the rat. *Pharmacol Biochem Behav* **14**: 595–601, 1981.
11. Commissaris, R. L., D. J. Mokler, W. H. Lyness, K. E. Moore and R. H. Rech. The behavioral effects of hallucinogens in rats following 5,7-dihydroxytryptamine administration into the medial forebrain bundle. *Pharmacol Biochem Behav* **14**: 915–918, 1981.
12. Commissaris, R. L. and R. H. Rech. Antagonism of the behavioral effects of 2,5-dimethoxy-4-methylamphetamine (DOM) and quipazine by metergoline. *Pharmacol Biochem Behav* **15**: 659–662, 1981.
13. Fertziger, A. P. and R. Fischer. Interaction between narcotic antagonist (naloxone) and lysergic acid diethylamide (LSD) in the rat. *Psychopharmacology (Berlin)* **54**: 313–314, 1977.
14. Henck, J. W., D. J. Mokler, R. L. Commissaris and R. H. Rech. Cyclazocine disruption of operant behavior is antagonized by naloxone and metergoline. *Pharmacol Biochem Behav* **18**: 41–45, 1983.
15. Holtzman, S. G. Stimulus properties of opioids with mixed agonist and antagonist activity. *Fed Proc* **41**: 2328–2332, 1982.
16. Joseph, J. A. and J. B. Appel. Behavioral sensitivity to LSD: Dependence upon the pattern of central 5-HT depletion. *Pharmacol Biochem Behav* **6**: 499–504, 1977.
17. Linton, M. and P. S. Gallo, Jr. *The Practical Statistician: Simplified Handbook of Statistics*. Monterey, CA: Brooks/Cole Publishing Co., 1975.
18. Malec, D. and R. Langwinski. Effect of quipazine and fluoxetine on analgesic-induced catalepsy and antinociception in the rat. *J Pharm Pharmacol* **32**: 71–73, 1980.
19. Martin, W. R., C. W. Gorodetzky and T. K. McClane. An experimental study in the treatment of narcotic addicts with cyclazocine. *Clin Pharmacol Ther* **7**: 455–465, 1966.
20. Mokler, D. J., R. L. Commissaris, M. R. Warner and R. H. Rech. Blockade of the behavioral effects of lysergic acid diethylamide, 2,5-dimethoxy-4-methylamphetamine, quipazine and lisuride by 5-hydroxytryptamine antagonists. *J Pharmacol Exp Ther* **227**: 557–562, 1983.
21. Rech, R. H., D. J. Mokler, R. L. Commissaris and J. W. Henck. Behavioral interactions of opioid agonists and antagonists with serotonergic systems. Presented at Forty-fifth Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc., Lexington, KY, 1983.

22. Ruffing, D., B. Kovacic, S. Demetrious and E. Domino. Naloxone enhancement of DMT and LSD-25 induced suppression of food-rewarded bar pressing behavior in the rat. *Psychopharmacology (Berlin)* **62:** 207–210, 1979.
23. Ruffing, D. M. and E. F. Domino. Effects of selected opioid agonists and antagonists on DMT- and LSD-25-induced disruption of food-rewarded bar pressing behavior in the rat. *Psychopharmacology (Berlin)* **75:** 226–230, 1981.
24. Ruffing, D. M. and E. F. Domino. Interaction of synthetic opioid metenkephalin peptide analogs, Lilly 127623 and FK 33-824 with indole hallucinogens: Antagonism of N,N-dimethyltryptamine and LSD-induced disruption of food-rewarded bar pressing behavior in the rat. *Psychopharmacology (Berlin)* **80:** 315–318, 1983.
25. Sparber, S. B. and H. A. Tilson. Environmental influences upon drug-induced suppression of operant behavior. *J Pharmacol Exp Ther* **179:** 1–9, 1971.
26. Winter, J. C. Effects of the phenethylamine derivatives BL-3912, fenfluramine, and Sch-12679, in rats trained with LSD as a discriminative stimulus. *Psychopharmacology (Berlin)* **68:** 159–162, 1980.