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Morphine-Like Stimulus Effects in the Monkey: Opioids with Antagonist Properties[1]

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SCHAEFER, G. J. AND S. G. HOLTZMAN. *Morphine-like stimulus effects in the monkey: Opioids with antagonist properties*. PHARMAC. BIOCHEM. BEHAV. **14**(2) 241–245, 1981.—The discriminative stimulus properties of opioids with a wide spectrum of agonist and antagonist properties were evaluated in squirrel monkeys trained to discriminate between morphine and saline in a two-choice discrete-trial avoidance task. Stimulus control was considered to be established when the monkeys reliably completed at least 22 trials of a 25-trial session on the lever appropriate for the drug state. Tests of stimulus generalization were conducted with compounds that were previously shown in the rat to produce discriminative stimulus effects that are: (a) morphine-like (profadol, WY-16,225, pentazocine, butorphanol, nalmexone); (b) cyclazocine-like (cyclazocine, ketocyclazocine, levallorphan); (c) neither morphine-like nor cyclazocine-like (nalbuphine and nalorphine). Profadol and WY-16,255 were equipotent with morphine in producing morphine-like stimulus effects. Naloxone antagonized the morphine-appropriate responding produced by all three compounds, but 10 times more naloxone was needed to block the stimulus effects of WY-16,255 than to block those of either morphine or profadol consistent with the known antagonist properties of WY-16,255. None of the other drugs produced complete morphine-like stimulus control of behavior but, with the exception of nalorphine, the highest dose of each resulted in about half of the trials being completed on the morphine-appropriate choice lever. These results confirm the heterogeneous nature of the discriminative stimulus effects of opioids with mixed agonist and antagonist properties and indicate the importance of interspecies comparisons.

Discriminative stimulus effects Morphine Squirrel monkeys Narcotic antagonists

IN a previous study [13] it was demonstrated that cyclazocine produced stimulus control of behavior in squirrel monkeys, and, recently, this finding has been extended to rats [16]. In both species, several opioids with mixed agonist and narcotic antagonist properties, such as ketocyclazocine and levallorphan, were shown to produce stimulus effects comparable to those produced by the training dose of cyclazocine, whereas other compounds with both agonist and antagonist activity, such as nalbuphine and nalorphine, did not. However, results were not always consistent between the two species. For example, pentazocine produced cyclazocine-like stimulus effects in rats, but not in squirrel monkeys. The mixed-acting narcotic agonist-antagonists have also been tested in rats trained to discriminate between morphine and saline [14,15]. Once again, the compounds fell into two broad groupings: those that produced morphine-like stimulus control of behavior (e.g., pentazocine, butorphanol, nalmexone), and those that did not (e.g., cyclazocine, levallorphan, nalorphine).

To date then, the mixed-acting narcotic agonist-antagonists have been tested in cyclazocine-trained rats and monkeys as well as in morphine-trained rats. In view of the heterogeneity of the discriminative stimulus effects of these compounds [13], and the well-known species differences in the behavioral effects of opioids [4], it was deemed important to extend the characterization of the stimulus properties of the mixed-acting narcotic agonist-antagonists to morphine-trained squirrel monkeys. Therefore, the primary purpose of this study was to evaluate the discriminative stimulus properties of drugs that encompass a range of morphine-like and cyclazocine-like agonist effects in squirrel monkeys trained to discriminate between saline and morphine. Two of the compounds that were tested, profadol and WY-16,225, produced stimulus effects comparable to those of the training dose of morphine. The stimulus effects of these drugs were further characterized by observing their sensitivity to naloxone blockade. The results of this study provide further confirmation of the heterogeneity of the discriminative

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stimulus effects of the mixed-acting narcotic agonist-antagonists, and indicate that cross-species comparisons with several test compounds is a fruitful approach to the classification of the stimulus effects of these opioids.

METHOD

Animals

The subjects were six adult squirrel monkeys (*Saimiri sciureus*), four females and two males. Four of the monkeys had been used in a previous study of the discriminative stimulus properties of morphine [12], and had been tested with a variety of drugs in the same manner as in the present study; the other two monkeys were experimentally naive. Between experiments, each monkey was housed in an individual cage in a colony room with food and water always available. The colony room was illuminated between 0700 and 1900 hours.

Apparatus

Experimental sessions were conducted with the monkey restrained in a small primate cockpit (BRS/LVE, Beltsville, MD) which was placed within a ventilated cubicle that was light-proof and sound-attenuating. Two brass electrodes rested gently on a shaved portion of the monkey's tail which was kept immobile by a Plexiglas stock. The brass electrodes were connected to an electric shock generator (catalog no. SG-903, BRS/LVE) that was capable of emitting an electric current of constant intensity. Two response levers (catalog no. CRL-005, BRS/LVE) were mounted 10 cm apart on the wall of the test chamber that faced the monkey. The animal was prevented from reaching both levers simultaneously by a Plexiglas barrier that was positioned between the animal and the levers. In order to press a lever, the monkey had to fully extend its arm through one of the two small openings (2.5×4.0 cm) that were in the right and left edges of the barrier.

Discrimination Training

The monkeys were trained in a discrete trial avoidance procedure to press one of the two levers in order to terminate a trial and avoid or escape from electric shocks delivered to the tail as described previously [12,13]. Illumination of the house light signaled the onset of a trial; beginning 5.0 sec later, a 3 mA electric shock was delivered to the tail in pulses of 1.0 sec duration every 2.0 sec. A response on the correct lever at any time during a trial immediately terminated the trial and produced a 50-sec intertrial interval in which the house light was off and the chamber was dimly illuminated by a yellow stimulus lamp situated at eye level between the two levers. A session ended after 25 trials had been completed or 40 min had elapsed.

Training sessions were conducted 4 days each week, on Mondays, Tuesdays, Thursdays, and Fridays. The monkeys were placed in the test chamber 15 min before each session and injected intramuscularly with either saline or 3.0 mg/kg of morphine in a double alternation sequence. The right choice lever was the correct lever for three of the monkeys on days when they received saline and the left choice lever was correct on days when they received morphine. The other three monkeys were trained under the opposite conditions. A response on the incorrect choice lever during a trial had no programmed consequences but the trial was recorded as incorrect. The behavior of the monkeys was considered to be under the stimulus control of saline and morphine when the monkeys could reliably complete the training sessions with at least 22 correct trials out of the 25.

Stimulus Generalization Tests

After stimulus control of behavior was established, tests of generalization to novel drug conditions were conducted on Tuesdays and Fridays provided that the monkey continued to meet the criterion of completing at least 22 out of 25 correct trials in training sessions held on Mondays and Thursdays. Both choice levers were electrically activated during drug test sessions so that a trial could be terminated by the first response on either lever. Test sessions and training sessions were the same in all other respects. Stimulus generalization (i.e., dose-response) curves for each drug were determined in three or four monkeys. In the determination of each curve, doses were tested in a random sequence that also incluced saline and 3.0 mg/kg of morphine. All drugs were injected intramuscularly 15 min before the first trial of the session.

Drugs

Morphine sulfate was obtained from Mallinkrodt Chemical Works (St. Louis, MO). The following drugs were generously donated: nalbuphine hydrochloride, nalmexone hydrochloride, naloxone hydrochloride (Endo Laboratories, Garden City, NY); cyclazocine base, ketocyclazocine base, pentazocine base (Sterling-Winthrop Research Institute, Rensselaer, NY); levallorphan tartrate (Roche Laboratories, Nutley, NJ); nalorphine hydrochloride (Merck and Company, Chemical Division, Rahway, NJ); butorphanol tartrate (Bristol Laboratories, Syracuse, NY); profadol hydrochloride (Warner-Lambert/Parke-Davis, Ann Arbor, MI); WY-16,225[(−) -13β-amino- 5,6,7,8,9,10,11,12-octohydro-5α-methyl-5,11-methanobenzocylodecen-31-ol hydrobromide] (Wyeth Laboratories, Radnor, PA). Cyclazocine, ketocyclazocine and pentazocine were dissolved in 8.5% lactic acid and 1.0 N sodium hydroxide in a 3:2 ratio. Butorphanol, nalbuphine and WY-16,225 were dissolved in distilled water; the remaining drugs were dissolved in 0.9% saline. All of the drugs were injected into the thigh muscle in a volume of 0.5 ml/kg of body weight. When naloxone was administered concomitantly with another drug, naloxone was injected first and the other drug was injected a few seconds later into a different part of the thigh muscle. Drug doses are expressed in terms of the free base.

Data Analysis

The data are presented as the mean number of trials completed on the morphine-appropriate lever. All trials not completed on the morphine-appropriate lever were completed on the vehicle-appropriate lever. A test drug was considered to produce stimulus control of behavior comparable to that produced by morphine (*viz.*, to substitute for the training dose of morphine) when an average of 22 out of 25 trials (i.e., 88%) by the group were completed on the morphine-appropriate lever.

For each session the cumulative latency to complete the 25 trials was also recorded. A randomized block design [8] analysis of variance was used to analyze the latency data. Significant F-ratios were further examined using Dunnett's test [2] to compare latency scores after vehicle and graded doses of drugs. Response latencies are expressed as the mean ± S.E.M.

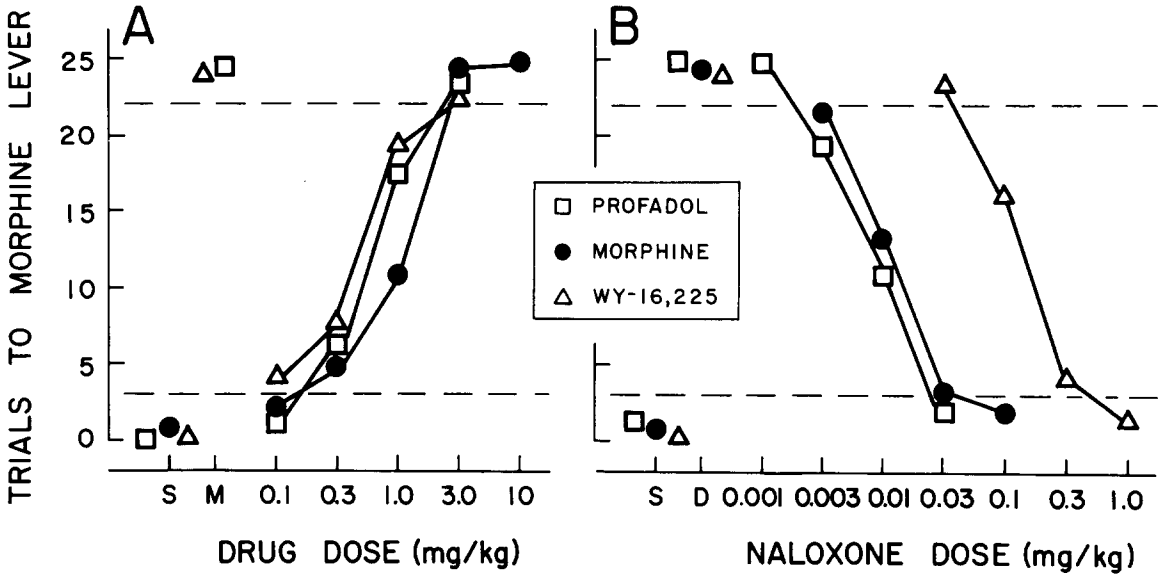

FIG. 1. Dose-response curves of morphine and two drugs that produce stimulus effects comparable to those of morphine (Panel A), and antagonism of the morphine-like stimulus effects of 3.0 mg/kg of morphine, profadol and WY-16,225 by concomitant administration of graded doses of naloxone (Panel B). Each point is the mean number of trials completed on the morphine-appropriate choice lever in a 25-trial session; the remaining trials of the session were completed on the saline-appropriate lever. The isolated points above "S" and "M" indicate the mean number of trials completed on the morphine-appropriate lever after an injection of saline or the training dose of morphine. The isolated points above "D" indicate the mean number of trials completed on the morphine-appropriate lever after an injection of 3.0 mg/kg of morphine, profadol or WY-16,225 in combination with saline. Means are based upon one observation in each of four monkeys. The upper and lower horizontal dashed lines indicate the minimum levels of discriminative responding at which the performance of the monkeys was maintained with the training dose of morphine and saline, respectively.

RESULTS

Figure 1A shows the stimulus generalization curves for morphine (0.1–10 mg/kg) and two additional opioids—profadol (0.1–3.0 mg/kg) and WY-16,225 (0.1–3.0 mg/kg). All three drugs produced dose-dependent increases in responding on the morphine-appropriate lever and were equipotent in producing morphine-like stimulus control of behavior. However, the stimulus control produced by these three drugs was not equally sensitive to antagonism by naloxone. The stimulus effects of morphine and profadol were completely blocked by 0.03–0.1 mg/kg of naloxone, but a 10-fold greater dose of naloxone (1.0 mg/kg) was needed to produce comparable blockade of the stimulus effects of WY-16,225 (Fig. 1B). The response latency scores were significantly affected by morphine, $F(5,15)=4.1$, $p<0.05$., and Dunnett's test indicated that this was due to an increase ($p<0.05$) following the administration of 10 mg/kg of morphine compared to saline administration (72 ± 21 vs. 33 ± 9 sec).

Three mixed-acting narcotic agonist-antagonist compounds which produced stimulus effects comparable to those of morphine in the rat [14,15]—pentazocine (0.1–3.0 mg/kg), butorphanol (0.003–3.0 mg/kg), nalmexone (0.1–3.0 mg/kg)—were tested. None of these compounds produced stimulus control comparable to morphine in the monkey up to the highest dose at which the animals could still complete the session (Fig. 2A). All three compounds produced dose-dependent increases in the number of trials completed on the morphine-appropriate lever which did not exceed an average of 14 for any of the drugs. Butorphanol produced the maximum of 14 responses on the morphine lever at 0.1 mg/kg; increasing the dose 30-fold higher did not result in a further increase in drug-appropriate responding.

Cyclazocine (0.01–0.3 mg/kg) and two drugs that produced cyclazocine-like stimulus control in the rat [16] and squirrel monkey [13]—ketocyclazocine (0.01–1.0 mg/kg), levallorphan (0.1–3.0 mg/kg)—also failed to produce morphine-like stimulus control (Fig. 2B). The three compounds produced dose-dependent increases in the average number of trials completed on the morphine-appropriate lever up to a maximum of 16.7. Cyclazocine produced a significant increase in the latency score, $F(4,8)=5.2$, $p<0.05$, which resulted from the effects of the highest dose (210 ± 81 vs 51 ± 14 sec; $p<0.05$). An almost 4-fold increase in the latency score occurred after 1.0 mg/kg of ketocyclazocine compared to the vehicle score (117 ± 48 vs 31 ± 14 sec), but the analysis of variance for this drug was not significant.

Nalorphine (1.0–30 mg/kg) and nalbuphine (1.0–30 mg/kg), drugs which produced neither morphine-like stimulus control in the rat [15] nor cyclazocine-like stimulus control in the squirrel monkey [13] or rat [16] similarly failed to produce stimulus effects comparable to the training dose of morphine (Fig. 2C). Nalbuphine produced a dose-related increase in the number of trials completed on the morphine-appropriate lever up to a maximum of 16. However, the animals responded only on the saline lever after each of the doses of nalorphine.

DISCUSSION

The evaluation of a series of opioid compounds with a wide spectrum of narcotic agonist and antagonist properties

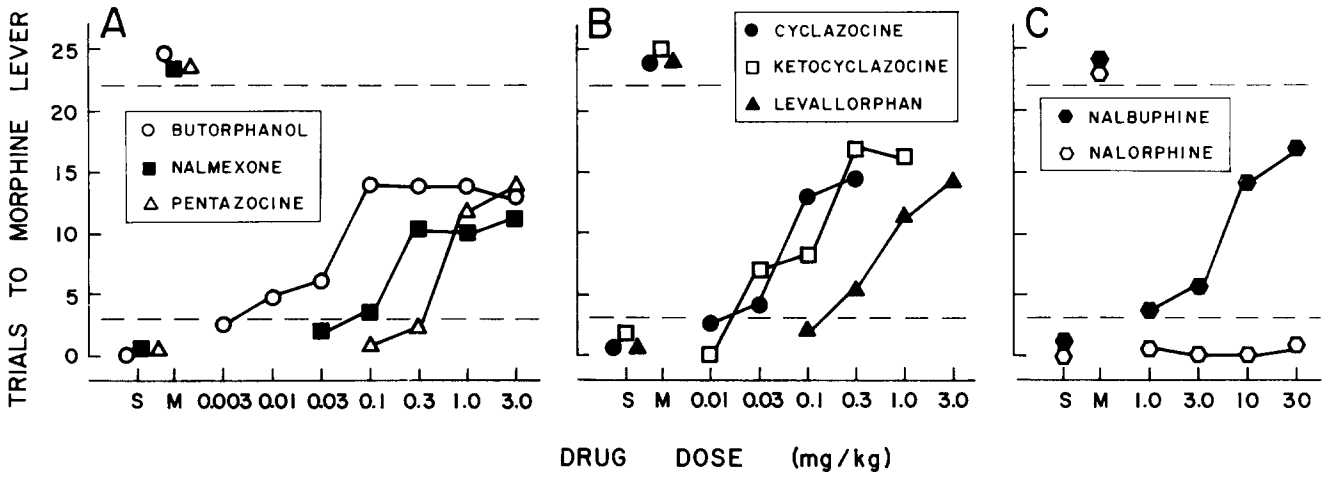

FIG. 2. Dose-response curves of drugs with mixed agonist and narcotic antagonist properties which were previously shown to produce discriminative stimulus effects in the rat that are: morphine-like (Panel A); cyclazocine-like (Panel B); neither morphine-like nor cyclazocine-like (Panel C). Each point represents a mean based upon one observation in each of four (butorphanol, levallorphan) or three monkeys. Other details are the same as in Fig. 1.

in morphine-trained squirrel monkeys extends previous drug discrimination studies in this species and in the rat. It is apparent from our data that there are species differences in the discriminative stimulus properties of the mixed-acting narcotic agonist-antagonists and that this group of drugs does not produce a homogenous set of stimulus effects in contrast to the morphine-like agonists [1, 12, 14, 15].

Both profadol and WY-16,225 produced stimulus control of behavior comparable to that produced by the 3.0 mg/kg training dose of morphine. However, the results of the naloxone antagonism experiments indicate that the three compounds are not completely similar to each other. Whereas the stimulus effects of morphine and profadol were antagonized by the same doses of naloxone, approximately 10-times as much naloxone was required to block the stimulus control by WY-16,225. The morphine-like stimulus control produced by profadol and its blockade by naloxone are consistent with its classification as a "pure" partial morphine agonist [6,7]. On the other hand, WY-16,225 was the only compound with antagonist activity that produced morphine-like stimulus control in the squirrel monkey; it has previously been found to produce morphine-like stimulus control in rats (Holtzman and Shannon, unpublished observations). Although WY-16,225 has been reported to have almost exclusively morphine-like agonist activity in rats and monkeys [11] its resistance to naloxone antagonism relative to morphine and profadol is characteristic of opioids that possess both agonist and antagonist properties [9].

The data presented here and in our previous report suggest that, in contrast to the rat, the squirrel monkey can discriminate between morphine-like and pentazocine-like drugs, at least in the case where morphine is used as the training drug. In the rat, pentazocine produced morphine-like stimulus control when morphine was the training drug [14], and conversely, morphine produced pentazocine-like stimulus control when pentazocine was the training drug [10]. Pentazocine also produced cyclazocine-like stimulus control in rats trained to discriminate cyclazocine from vehicle [16]. A different pattern emerged from the experiments using monkeys. Pentazocine did not generalize to cyclazocine in squirrel monkeys trained in a saline-cyclazocine discrimination [13], nor did it generalize to morphine in the studies reported here. Consistent with this finding are the recent reports that pentazocine did not generalize completely to etorphine, a potent narcotic agonist, in rhesus monkeys trained to discriminate between etorphine and its vehicle [17], or to morphine in pigeons trained to discriminate between saline and morphine [3]. In the rat nalmexone was equipotent to morphine in producing morphine-like stimulus control [15], but it did not substitute for morphine in the squirrel monkey. The failure to produce morphine-like stimulus control of behavior with pentazocine and nalmexone may have been due to our inability to test sufficiently high doses. However, dosage was not a limiting factor in the case of butorphanol. Although butorphanol was 10-times more potent than morphine in producing morphine-like stimulus control of behavior in the rat [15], butorphanol did not produce morphine-like stimulus control in the squirrel monkey, even though it was tested over a dose range in which a plateau in responding was clearly reached. The observation that butorphanol did generalize to cyclazocine in squirrel monkeys [13] suggests that its discriminative effects more closely resemble those of cyclazocine than those of morphine in this species.

Ketocyclazocine and cyclazocine were previously found to be equipotent in producing cyclazocine-like stimulus control in the monkey, and levallorphan was $^1/_3$ as potent as cyclazocine [13]. Although all three of these drugs produced dose-related increases in responding on the morphine-appropriate lever in the present studies, no substitution for morphine occurred even at the highest dose where the increased latencies suggested the onset of behavioral disruption. Qualitatively similar results were obtained with these three drugs in rats trained to discriminate between saline and morphine [14,15].

Nalbuphine failed to generalize either to morphine in the rat [15] or to cyclazocine in the squirrel monkey [13]. In these experiments using morphine-trained squirrel monkeys, nalbuphine did not produce morphine-like stimulus control. Similarly, nalorphine did not produce morphine-like stimulus control in the squirrel monkey; it also failed to produce morphine-like stimulus control in the rat [15] and it failed to

produce cyclazocine-like discriminative effects either in the rat [16] or in the squirrel monkey [13]. After nalorphine administration in the present study, the squirrel monkeys responded almost exclusively on the saline-appropriate lever, an outcome previously obtained only with naloxone [12]. The results of the present study together with earlier data from our laboratory [12–16] and with the data from other groups (e.g., [3,17]) indicate the need for interspecies comparisons of the discriminative stimulus effects of opioids having mixed agonist and narcotic antagonist properties.

REFERENCES

1. Colpaert, F. C. Discriminative stimulus properties of narcotic analgesic drugs. *Pharmac. Biochem. Behav.* **9:** 863–887, 1978.
2. Dunnett, C. W. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50:** 1096–1121, 1955.
3. Herling, S., E. H. Coale, R. J. Valentino, D. W. Hein and J. H. Woods. Narcotic discrimination in pigeons. *J. Pharmac. exp. Ther.* **214:** 139–146, 1980.
4. Jaffe, J. H. and W. R. Martin. Narcotic analgesics and antagonists. In: *The Pharmacological Basis of Therapeutics,* edited by L. S. Goodman and A. Gilman. New York, New York: MacMillian Publishing Co., 1975, pp. 245–283.
5. Jasinski, D. R., J. D. Griffith, J. S. Pevnick and S. C. Clark. Progress report on studies from the Clinical Pharmacology Section of the Addiction Research Center. Reported, Committee on Problems of Drug Dependence, 1975, 121–161.
6. Jasinski, D. R., W. R. Martin and R. Hoeldtke. Studies of the dependence-producing properties of GPA-1657, profadol and propiram in man. *Clin. Pharmac. Ther.* **12:** 613–649, 1971.
7. Jasinski, D. R. and J. C. Nutt. Progress report on the clinical assessment program of the Addiction Research Center. Reported, Committee on Problems of Drug Dependence, 1973, 108–130.
8. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences.* Belmont, California: Brooks/Cole, 1968.
9. Kosterlitz, H. W., J. A. H. Lord and A. J. Watt. Morphine receptor in the myenteric plexus of the guinea pig ileum. In: *Agonist and Antagonist Actions of Narcotic Analgesic Drugs,* edited by H. W. Kosterlitz, H. O. J. Collier and J. E. Villarreal. Baltimore: University Park Press, 1973, pp. 45–61.
10. Kuhn, D. M., I. Greenberg and J. B. Appel. Stimulus properties of the narcotic antagonist pentazocine: Similarity to morphine and antagonism by naloxone. *J. Pharmac. exp. Ther.* **196:** 121–127, 1976.
11. Malis, J. L., M. E. Rosenthale and M. I. Gluckman. Animal pharmacology of WY-16,225, a new analgesic agent. *J. Pharmac. exp. Ther.* **194:** 488–498, 1975.
12. Schaefer, G. J. and S. G. Holtzman. Discriminative effects of morphine in the squirrel monkey. *J. Pharmac. exp. Ther.* **201:** 67–75, 1977.
13. Schaefer, G. J. and S. G. Holtzman. Discriminative effects of cyclazocine in the squirrel monkey. *J. Pharmac. exp. Ther.* **205:** 291–301, 1978.
14. Shannon, H. E. and S. G. Holtzman. Evaluation of the discriminative effects of morphine in the rat. *J. Pharmac. exp. Ther.* **198:** 54–65, 1976.
15. Shannon, H. E. and S. G. Holtzman. Further evaluation of the discriminative effects of morphine in the rat. *J. Pharmac. exp. Ther.* **201:** 55–66, 1977.
16. Teal, J. J. and S. G. Holtzman. Discriminative stimulus effects of cyclazocine in the rat. *J. Pharmac. exp. Ther.* **212:** 368–376, 1980.
17. Woods, J. H., S. Herling, R. J. Valentino, D. W. Hein and E. H. Coale, Jr. Narcotic drug discriminations by rhesus monkeys and pigeons. Proceedings of the 41st Annual Scientific Meeting, the Committee on Problems of Drug Dependence, Inc. *NIDA Research Monograph 27,* edited by L. S. Harris. Washington, D.C.: DHEW, 1979, pp. 128–134.