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Behavioral Effects of Systemically Administered Mu and Kappa Opioid Agonists in the Squirrel Monkey: Peptides Versus Alkaloids

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JONES, D. N. C. AND S. G. HOLTZMAN. *Behavioral effects of systemically administered mu and kappa opioid agonists in the squirrel monkey: Peptides versus alkaloids.* PHARMACOL BIOCHEM BEHAV 47(3) 421–426, 1994.—This study compared the effects of receptor-selective peptide and nonpeptide opioid agonists administered intramuscularly to squirrel monkeys responding under a fixed-interval 3-min schedule of stimulus termination. The mu opioid receptor agonist morphine (0.1–3.0 mg/kg) increased response rate at low doses and decreased it and quarter-life at higher doses. [D-Ala^2,N-Me-Phe^4,Gly-ol]Enkephalin (DAMGO; 0.3–3.0 mg/kg) reduced quarter-life at the highest dose. The kappa opioid receptor agonist U50,488H (0.1–1.0 mg/kg) elevated response rate transiently and dose-dependently decreased quarter-life. Dynorphin A(1–13) (0.3–10 mg/kg), a purported endogenous ligand of the kappa opioid receptor, decreased response rate slightly but significantly at 3.0 mg/kg and had no effect on quarter-life. Thus, the behavior of squirrel monkeys was affected by systemically administered peptide as well as by nonpeptide opioid drugs. The two alkaloids were much more effective than the two peptides, presumably because of greater ability to penetrate the blood-brain barrier. Quarter-life was often a more sensitive measure of drug effects than was response rate.

Receptor-selective opioids Opioid peptides Morphine Dynorphin A(1–13) Schedule-controlled behavior Stimulus termination Squirrel monkey

PEPTIDES that are synthesized and released in the periphery can mediate the transfer of information across the blood–brain barrier by direct as well as by indirect mechanisms (3). Peptides that are administered systemically can cross the blood–brain barrier by both saturable and nonsaturable transport (3), and can exert a broad range of effects upon central nervous system function (20). For example, very low doses of systemically administered opioid peptides are active in rodent models of learning and memory (10,11). However, the range of conditions under which behavioral effects of systemically administered opioid peptides have been studied remains limited.

In the present study, we compared the effects of prototypic peptide and nonpeptide opioid agonists administered intramuscularly on behavior of squirrel monkeys maintained under a fixed-interval schedule of stimulus termination. Morphine, the proteotypic mu opioid agonist, was compared with [D-Ala^2,N-Me-Phe^4,Gly-ol] enkephalin (DAMGO), a peptide with high selectivity for the mu opioid receptor (8), and U50,488H, a highly selective kappa opioid agonist (8), was compared with dynorphin A(1–13), the proposed endogenous ligand of the kappa opioid receptor (5).

METHOD

Subjects

Four male, experimentally naive squirrel monkeys (*Saimari sciureus*), weighing 735–1055 g, were used in this study (designated S69, S81, S82, and S86). Two animals were housed

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individually (S69 and S82) and the other two animals (S81 and S86) were housed as a pair. Each animal had free access to food (Purina Monkey Chow 5040) and water in its home cage. The diet was supplemented with fresh fruit twice weekly, peanuts twice weekly, and a vitamin supplement three times per week.

Apparatus

During an experimental session, each monkey was restrained in a small primate cockpit (No. 142-11: BRS/LVE, Laurel, MD), which was housed in a well-ventilated, sound-attenuating chamber provided with constant white masking noise. The chamber was illuminated by a house light mounted on the front panel of the cockpit. Two brass electrodes connected to a shock generator (SG 903, BRS/LVE) rested on a cleanly shaved portion of the monkey's tail, which was held immobile by a Plexiglas stock. Two response levers (121-05, BRS/LVE) were mounted 10 cm apart on the wall of the test chamber facing the monkey. Illumination of green cue lights located 8.5 cm above the response levers served as the discriminative stimulus. Events were controlled and data recorded by a microcomputer.

Procedure

Monkeys were trained under a fixed-interval (FI) 3-min schedule of stimulus termination (13) with a 3.0-s limited hold. The house light and the green cue light above the right lever illuminated the chamber during the 3-min interval and, after 3 min elapsed, the subject had 3.0 s to press the right lever and terminate the lights that were associated with the impending electrical stimulus. When the animal pressed the lever during the limited hold time and avoided the stimulus, a 60-s time-out period followed, during which the chamber was darkened and responses had no scheduled consequences. In the absence of a response during the 3.0-s period, a 4.0–5.0 mA stimulus of 250 ms duration was delivered every 5.0 s until either the monkey made a correct response or five stimuli were delivered, after which a 60-s time-out occurred. A session consisted of 30 consecutive FI 3-min components, each followed by a 60-s time-out.

After performance had stabilized, the effects of morphine (0.1–3.0 mg/kg), DAMGO (0.3–3.0 mg/kg), dynorphin A (0.3–10 mg/kg), and U50,488H (0.1–1.0 mg/kg) were determined. The doses of each drug were administered in a random sequence that also included the drug vehicle. Drugs were administered into the thigh muscle as a 15-min pretreatment.

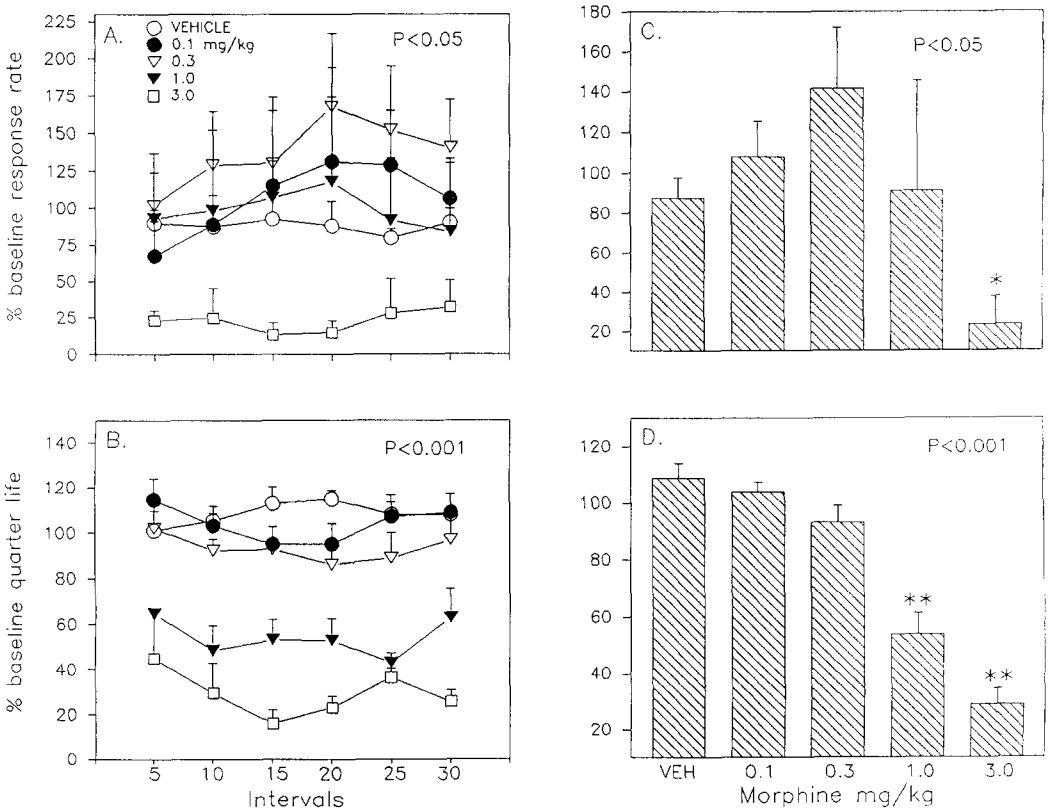

FIG. 1. Effects of morphine (0.1–3.0 mg/kg) and vehicle on response rate (A,C) and quarter-life (B,D) in squirrel monkeys responding under a FI 3-min schedule of stimulus termination. Data were transformed to a percent of values on the day that preceded each drug test (% baseline) and are presented as means ± SEM (n = 3) for blocks of five intervals (A,B) and for the entire 30-interval session (C,D). The p values are shown for main effect of morphine treatment.

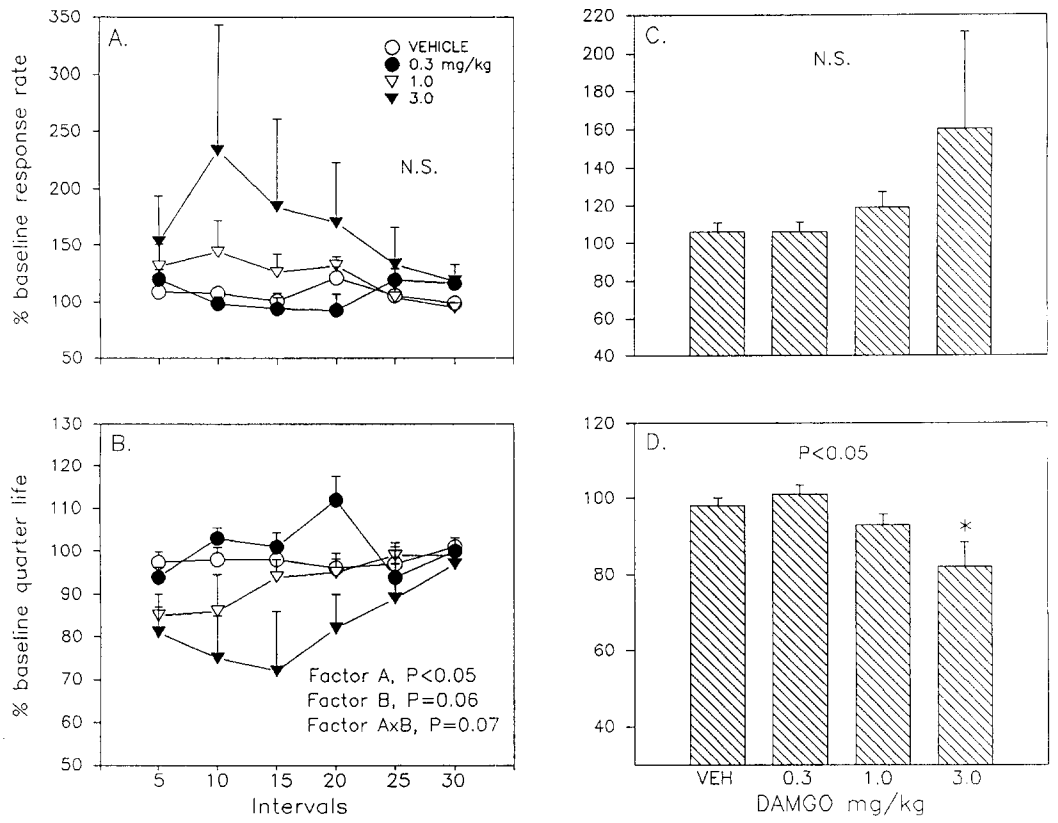

FIG. 2. Effects of DAMGO (0.3–3.0 mg/kg) and vehicle on response rate (A,C) and quarter-life (B,D) in squirrel monkeys (n = 4) responding under a FI 3-min schedule of stimulus termination. The p values are shown for effect by dose (factor A), by block of five intervals (factor B), and for the interaction of dose × block of intervals (factor A × B); N.S. = not statistically significant. Other details as in Fig. 1.

Experimental sessions were conducted daily, five to six times a week. The performance on the day before a drug-treatment day was used as the baseline level of performance. At least 2 to 3 days separated each drug test.

Drugs

Morphine sulfate was obtained from Penick Corporation (Newark, NJ), U50,488H from RBI (Natick, MA), and DAMGO from Bachem California (Torrance, CA). Dynorphin A(1-13) was obtained form Dr. N. M. Lee (Department of Pharmacology, University of Minnesota). All compounds were dissolved in distilled water except for morphine, which was dissolved in 0.9% saline.

Data Analysis

The response rate and quarter-life (the time required for the animal to make 25% of total responses for each interval) were calculated for each 180-s FI. The means for blocks of five intervals were calculated for each animal. The data are expressed as the percentage of the corresponding baseline data for the experimental session on the day preceding the test day (baseline control). The data for blocks of five intervals were subjected to two-factor (pretreatment dose = factor A; interval = factor B) analysis of variance (ANOVA) with repeated measures on both factors. The mean response rate and quarter-life for all 30 intervals of a session were subjected to one-factor ANOVA with repeated measures. Where appropriate, post hoc analysis was performed using Student's t-test corrected for multiple pairwise comparisons.

RESULTS

The responding during nondrug experimental sessions on the days preceding drug treatment was characteristic of normal performance maintained under FI schedules in squirrel monkeys. The response rate was very low during the early part of the interval but increased as the interval elapsed. Typically, 75% percent of all responses were made in the last 50–80 s of the interval. Individual mean rates of responding for all 30 intervals (±SD) on the control test day were 48 ± 11 (S69), 13 ± 3.3 (S82), 14 ± 3.5 (S86), and 10 ± 2.0 (S81) presses/min; mean quarter-life was 106 ± 12.1 (S69), 124 ± 7.2 (S82), 118 ± 7.9 (S86), and 118 ± 9.9 (S81) s. Monkeys were very successful in terminating the stimulus and received a tail shock very infrequently.

Morphine was tested in only three of the four monkeys (S69, S86, and S81). Administration of morphine significantly influenced response rate, $F(4, 8) = 5.12$, $p < 0.05$ (Fig. 1A and C); the effects appeared to be biphasic in nature. Lower doses of morphine (0.1 and 0.3 mg/kg) caused an increase in responding over the full session (Fig. 1C), which reached 140% of control after 0.3 mg/kg. However, this did not reach significance for data over the whole session because of the

variability between monkeys. Analysis of the data for blocks of five intervals showed a main effect of morphine treatment, $F(4, 8) = 5.1$, $p < 0.05$; 0.3 mg/kg raised response rate significantly from the 15th to the 25th interval ($p < 0.05$, Fig. 1A). The highest dose tested, 3.0 mg/kg, significantly reduced the response rate ($p < 0.05$, Fig. 1C), an effect that lasted for the duration of the session ($p < 0.05$–0.01, Fig. 1A).

Morphine reduced the quarter-life markedly and dose dependently, $F(4, 8) = 35.42$, $p < 0.001$ (Fig. 1B and D); post hoc analysis revealed significant differences following 1.0 and 3.0 mg/kg ($p < 0.01$). These effects lasted for the duration of the experiment (Fig. 1B).

Administration of DAMGO had no significant effects upon response rate (Fig. 2A and C), although there appeared to be a dose-dependent trend towards elevated response rates during the first half of the test session (Fig. 2A). DAMGO reduced quarter-life significantly, $F(3, 9) = 4.05$, $p < 0.05$ (Fig. 2D); post hoc analysis revealed a significant difference from vehicle pretreatment following 3.0 mg/kg ($p < 0.05$). Analysis of the data for blocks of intervals showed a significant effect of pretreatment, $F(3, 9) = 4.35$, $p < 0.05$ (Fig. 2B); quarter-life following 3.0 mg/kg was significantly lower than that following vehicle pretreatment for the first 20 intervals, whereupon it returned to vehicle control levels ($p < 0.05$–0.01, Fig. 2D); this was reflected in a near significant main effect of intervals, $F(5, 15) = 2.72$, $p = 0.061$. Post hoc analysis also revealed a small elevation in quarter-life following 0.3 mg/kg DAMGO during the fourth block of intervals ($p < 0.05$, Fig. 2D).

U50,488H had no significant effects upon the overall response rate (Fig. 3C). Analysis of the data for blocks of intervals showed a near significant effect of pretreatment, $F(3, 9) = 3.18$, $p = 0.077$; the response rate was raised following 0.1 mg/kg for the first block of intervals only ($p < 0.01$) and following 0.3 mg/kg for the second and final block of intervals ($p < 0.05$, Fig. 3A). U50,488H reduced the quarter-life dose dependently, $F(3, 9) = 45.9$, $p < 0.0001$ (Fig. 3D); mean quarter-life was significantly different from vehicle controls following 0.3 and 1.0 mg/kg ($p < 0.01$). Analysis of the data for blocks of intervals revealed significant main effects of pretreatment, $F(3, 9) = 46.6$, $p < 0.0001$, and of intervals, $F(5, 15) = 4.2$, $p < 0.014$ (Fig. 3B). The U50,488H-induced decrease in quarter-life was greatest during the first five intervals following all doses tested. One out of four monkeys (S82) vomited after receiving 0.3 mg/kg U50,488H, and three out of four monkeys (S81, S82, and S86) vomited after receiving 1.0 mg/kg. Therefore, a higher dose of U50,488H was not tested.

Dynorphin A(1-13) significantly influenced response rate, $F(4, 12) = 3.48$, $p < 0.005$ (Fig. 4C); post hoc anlaysis revealed a significant reduction compared with vehicle treatment following 3.0 mg/kg ($p < 0.005$). Analysis of the data for blocks of intervals showed a near significant influence of dynorphin A(1-13) upon response rates, $F(4, 12) = 3.14$, $p =$

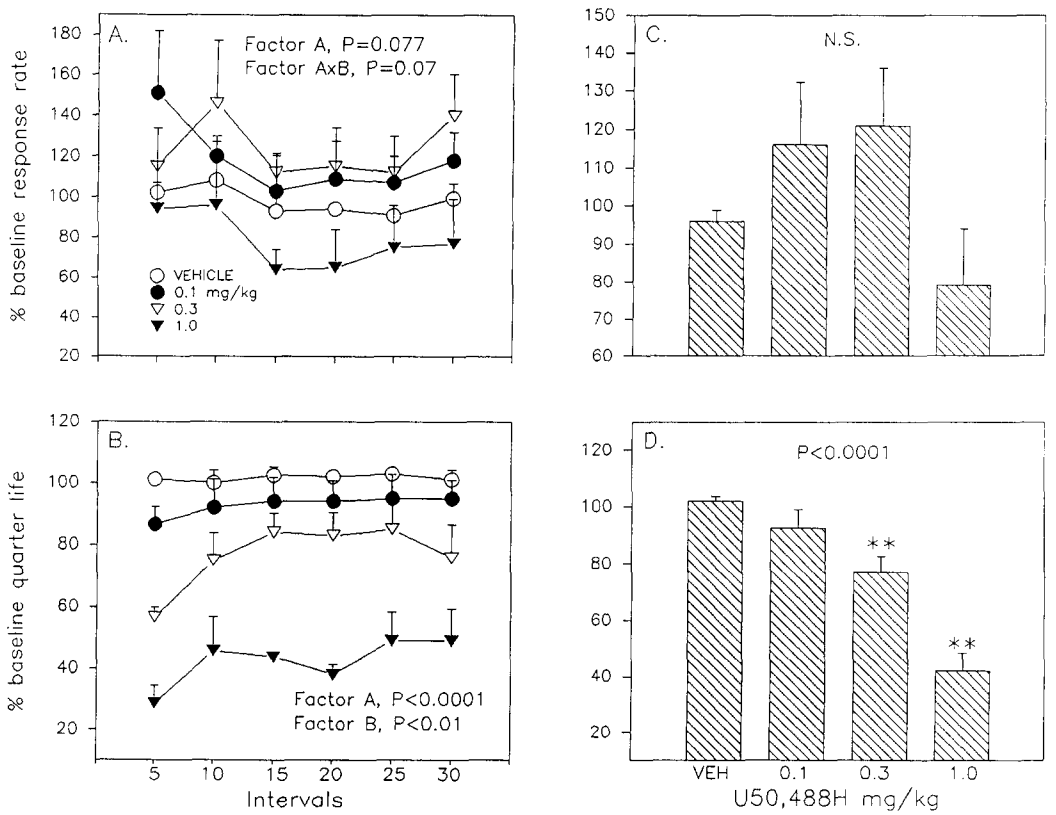

FIG. 3. Effects of U50,488H (0.1–1.0 mg/kg) and vehicle on response rate (A,C) and quarter-life (B,D) in squirrel monkeys ($n = 4$) responding under a FI 3-min schedule of stimulus termination. Other details as in Figs. 1 and 2.

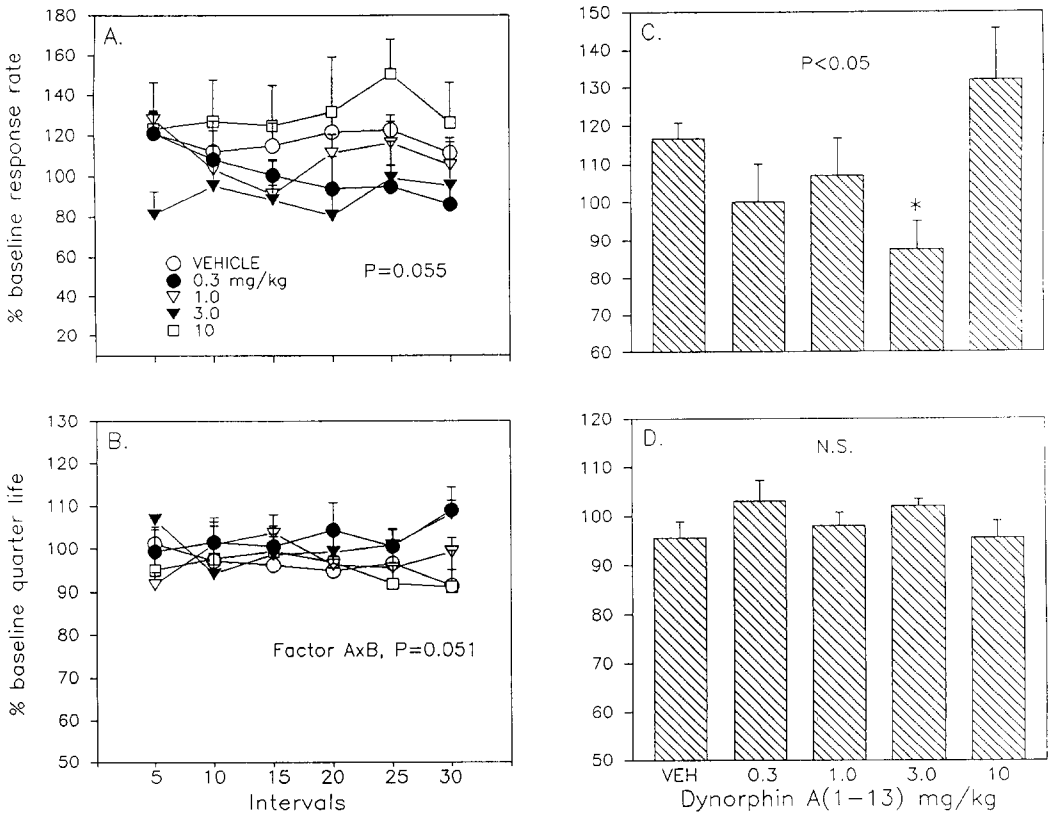

FIG. 4. Effects of dynorphin A(1-13) (0.3-10 mg/kg) and vehicle on response rate (A,C) and quarter-life (B,D) in squirrel monkeys (n = 4) responding under a FI 3-min schedule of stimulus termination. Other details as in Fig. 1 and 2.

0.055 (Fig. 4A); the reduction following 3.0 mg/kg dynorphin A(1-13) persisted over the length of the test session. The highest dose of dynorphin A(1-13), 10 mg/kg, tended to increase rate of responding (Fig. 4A and C). Dynorphin A(1-13) had no significant effects upon quarter-life (Fig. 4B and D).

DISCUSSION

The main finding of this study was that the behavior of squirrel monkeys maintained by a FI 3-min schedule of stimulus termination was affected by systemically administered peptide as well as by nonpeptide opioid drugs. However, the effects of the two opioid alkaloids, morphine and U50,488H, were much greater than those of the two opioid peptides, DAMGO and dynorphin A(1-13). This probably reflects the greater ability of the alkaloids to penetrate the blood-brain barrier in sufficient concentration to induce behavioral changes. For example, 1-2% of a systemically administered dose of morphine reaches the brain compared to less than 0.1% of a dose of the opioid pentapeptide cyclic[D-Pen^2,D-Pen^5]enkephalin (14,19).

Morphine had a biphasic effect on response rate: increasing it slightly at low doses and decreasing it by approximately 75% at the highest dose. This pattern is similar to what has been reported previously for morphine in squirrel monkeys responding under schedules of shock avoidance or shock presentation (9,12). Quarter-life, on the other hand, only decreased after morphine administration. In contrast to morphine, DAMGO had no significant effect on response rate, although a trend upward was evident at the highest dose, and it decreased quarter-life slightly but significantly. This pattern suggests that a higher dose might have resulted in the overall effects of DAMGO resembling those of morphine. However, cost became a limiting factor in escalating the dosage. In contrast to these minimal effects of DAMGO administered IM, DAMGO administered ICV to rats responding for food under a FI 3-min schedule was fully two orders of magnitude more potent than morphine in decreasing response rate, with an ED_{50} of 21 ng (2).

Like DAMGO, U50,488H had only marginal effects on rate of responding while decreasing quarter-life significantly. The impotency of U50,488H on the former measure might have been a consequence of the nature of the event-maintaining behavior. U50,488H is severalfold less potent in squirrel monkeys in decreasing responding maintained under schedules of stimulus termination than it is in decreasing food-maintained responding (4,6).

Dynorphin A(1-13) had minimal effects on behavior in this study. It decreased response rate significantly but only slightly at one dose, but not at the next higher or lower dose. Moreover, it was the only one of the four drugs that did not affect quarter-life. Doses of dynorphin A(1-13) as high as 30 μg ICV had no effect on response rates of rats under a FI 3-min schedule of food reinforcement (2). Despite its seemingly low efficacy on schedule-controlled behaviors, dynorphin A(1-13) can produce behavioral changes following systemic adminis-

tration, particularly where the actions of another drug are involved. For example, dynorphin A(1-13) administered IV reduced the behavioral manifestations of morphine withdrawal in morphine-dependent rats (7) and rhesus monkeys (1), and suppressed the expression of tolerance to and withdrawal from morphine in mice (16); injected IP, it reduced cocaine-induced motor activity in mice (17). Although dynorphin A(1-13) may function as an endogenous ligand of the kappa opioid receptor (5), its spectrum of activity is quite distinct from that of prototypical alkaloid kappa opioid receptor agonists, such as U50,488H. This raises the possibility that dynorphin A(1-13) has physiologically important neuromodulatory actions that are mediated at sites other than the traditional opioid receptors (15).

With the exception of the highest dose of morphine, drug effects on rate of responding were small or not detectable. In contrast to the relative insensitivity of that variable, quarter-life was a sensitive measure of drug effects on behavior. It was affected in an orderly manner by three of the four drugs examined.

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