Pharmacology Biochemistry & Behavior, Vol. 39, pp. 487–493. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Onset of Tolerance to Discriminative Stimulus Effects of Morphine[1]

ALICE M. YOUNG,[2,3] ELIZABETH S. STEIGERWALD, MALATH M. MAKHAY AND GEORGIOS KAPITSOPOULOS

Department of Psychology, Wayne State University, Detroit, MI 48202

Received 9 January 1991

YOUNG, A. M., E. S. STEIGERWALD, M. M. MAKHAY AND G. KAPITSOPOULOS. *Onset of tolerance to discriminative stimulus effects of morphine.* PHARMACOL BIOCHEM BEHAV **39**(2) 487–493, 1991.—Experiments assessed the onset of tolerance to discriminative stimulus effects of morphine in rats treated repeatedly with twice daily doses of 10 mg/kg morphine. Saline and 3.2 mg/kg morphine were established as discriminative stimuli for food-reinforced fixed-ratio performances in several groups of rats, and initial ED_{50} values were determined for stimulus and rate-altering effects of morphine. To assess onset of tolerance, training was halted and 10 mg/kg doses of morphine were administered repeatedly at 12-h intervals. In separate experiments, ED_{50} values were redetermined after various treatment periods. One treatment with 10 mg/kg morphine did not alter the ED_{50} for stimulus effects of morphine, whereas treatment for one or three days increased the ED_{50} by approximately 2-fold. Comparisons with published data showed even greater tolerance when treatment lasted one or two weeks. Tolerance to stimulus effects of morphine generally was accompanied by tolerance to its rate-decreasing effects. Repeated treatment with morphine also produced cross-tolerance to morphine-like stimulus effects of methadone and buprenorphine. As with morphine itself, greater tolerance developed with longer treatment. These results suggest that tolerance to discriminative stimulus effects of morphine develops gradually, with magnitude of tolerance increasing as a function of treatment duration.

Behavior Buprenorphine Discriminative stimulus Methadone Morphine μ Opioids Rats Tolerance

MORPHINE, the prototypic μ opioid, is readily established as a discriminative stimulus for operant behavior, and the doses required for stimulus control often show little variation over extended periods of regular discrimination training and its correlated drug exposure (4,27). However, if discrimination training contingencies are suspended during extended treatment with high doses of morphine, tolerance readily develops to stimulus effects of morphine (19, 22, 28). Such tolerance appears to arise from joint actions of pharmacodynamic and conditioning processes. Repeated treatment with high doses of morphine increases the dose required for generalization in a dose-dependent fashion, without a decrease in the maximal effect obtained (19, 21, 22, 28). This loss of sensitivity does not occur when subjects are treated with saline for equivalent periods (21,28), indicating that the interruption in training is not, in itself, responsible for altered sensitivity to stimulus effects of morphine. Sensitivity recovers quickly after treatment ends, without resumption of training, suggesting that loss of sensitivity after extended treatment does not result from transfer of control to a higher training dose [cf. (4)], since the effects of such retraining would be expected to persist after treatment ends. Additionally, the loss of sensitivity does not reflect a general loss of stimulus control, inasmuch as similar treatment regimens do not alter sensitivity to stimulus effects of a nonopioid such as cocaine (26).

Work by a number of investigators suggests that tolerance to stimulus effects of morphine and other μ opioids is dose and time dependent, pharmacologically specific, reversible, and sensitive to behavioral factors (8, 21, 22, 25, 28). Magnitude of tolerance increases as a function of treatment dose. In rats trained to discriminate saline and a dose of 3 mg/kg morphine, daily treatment with the training dose does not change the dose required for stimulus control, whereas extended treatment with doses of 10, 20 or 36 mg/kg/day increases the dose required for stimulus control by 2-, 4-, or 5-fold, respectively (21, 22, 28). In rats trained with higher doses of 5.6 or 10 mg/kg morphine, treatment doses of 20 or 110 mg/kg/day increase the dose required for stimulus control by only 2- or 4-fold (19,28), suggesting that the ratio between training and treatment doses also may control magnitude of tolerance. Tolerance to stimulus effects of morphine appears pharmacologically specific, as it is produced by repeated treatment with morphine, fentanyl or buprenorphine, but not by treatment with pentobarbital (8, 16, 19, 22, 28), and can be accompanied by marked cross-tolerance to stimulus effects of other mu agonists (8, 22, 29).

The present experiment examined how rapidly tolerance develops to stimulus effects of morphine. In previous experiments

[1]This work was supported by U.S. Public Health Service grant DA03796 from the National Institute on Drug Abuse.

[2]Recipient of Research Scientist Development Award K02 DA00132 from the National Institute on Drug Abuse.

[3]Requests for reprints should be addressed to Alice M. Young, Ph.D., Department of Psychology, Wayne State University. 71 West Warren Avenue, Detroit, MI 48202.

in rats trained with 3.2 mg/kg morphine, one or two weeks of treatment with twice daily injections of 10 mg/kg morphine increased the dose of morphine required for stimulus control by 3- or 4-fold, respectively (28). Comparisons with previous studies using different discrimination procedures suggested that this magnitude of tolerance was greater than that produced by shorter treatment periods [cf. (8,22)]. In order to examine the onset of tolerance more fully, the present study compared changes in sensitivity following either a single injection of 10 mg/kg morphine or treatment for one to three days with repeated doses of 10 mg/kg morphine, with previously reported changes after one and two weeks of treatment. In order to explore the generality of any time-dependent effects, other experiments examined cross-tolerance to morphine-like stimulus effects of methadone and buprenorphine after a more limited range of treatment periods. Changes in sensitivity to buprenorphine were compared after three days or two weeks of treatment, and changes in sensitivity to methadone after three days of treatment were compared to changes previously reported after one or two weeks of treatment (29).

METHOD

Subjects

Male Sprague-Dawley rats were housed individually in a colony room maintained under a 12-h light:dark cycle. Before training, subjects were reduced to 90% of initial free-feeding weights by restricting access to food. Thereafter, each subject received 13–16 g of Purina rat chow 30 to 90 min after each session and at mid-day when sessions were not conducted. Water was freely available in the home cage.

Apparatus

Experiments were conducted in chambers housed in ventilated, sound-attenuating cubicles. One wall of each chamber contained stimulus lamps, a recessed food receptacle centered 2 cm above the floor, and two response levers mounted 7 to 8 cm above the floor on either side of the food receptacle. Each press of a lever with a minimal downward weight of 28–35 g was recorded as a response. Food pellets (45 mg; PJ Noyes, Inc.) were delivered by a pellet dispenser mounted outside the chamber. Experimental contingencies were arranged and data recorded by microprocessors. White noise was present in the experimental room.

Procedure

Saline and a dose of 3.2 mg/kg morphine were established as discriminative stimuli for food-reinforced responses in five groups of subjects (Table 1). After training and initial tests of stimulus control, subjects in Group A were exposed to three treatment regimens of different durations. Subjects in Groups B and C had been studied previously under 2-week treatment regimens (28,29) and are included here for comparison with shorter regimens. In the present experiments, subjects in Group C were exposed to a 3-day treatment regimen, and subjects in Groups D and E were exposed to a 3-day and a 2-week regimen, respectively.

Training sessions were divided into three trials, each consisting of a 15-min timeout (TO) component, during which the chamber was dark and responses had no programmed consequences, followed by a 5-min ratio component. At the start of each trial, the rat was administered an injection of saline or morphine and placed in the darkened chamber. At the end of the TO component, the stimulus lamps were illuminated and responses were reinforced under a fixed-ratio (FR) schedule of food delivery. Following saline administration, responses on the right lever produced food; following morphine administration, responses on the left lever produced food. Each response on the incorrect lever reset the ratio counter to 0. The response requirement was increased gradually until 15 consecutive responses on the correct lever were required for food delivery. A trial ended, and the chamber was darkened, after 5 min or delivery of 50 pellets, whichever occurred first. At the end of 5 min, the subject was removed and administered an injection, and the next TO component was initiated. The sequence of trials was varied so that a similar number of drug and saline training trials were conducted each week. Sessions that began with a drug training trial consisted of three trials during which responses were reinforced on the drug-appropriate lever, with saline administered before the second and third trials. Sessions that began with a saline training trial consisted of three saline trials; two saline trials and one drug trial; or one saline trial and two drug trials, with saline administered before the final drug trial.

Initial tests of stimulus control. Training sessions were conducted five to seven days per week until the following criteria for discriminative control were met for five consecutive sessions: 1) the total number of responses emitted prior to the first reinforcer was less than 30, and 2) at least 90% of the total session responses were emitted on the injection-appropriate lever. After stimulus control was achieved, subjects were tested under a cumulative dosing procedure with morphine and, for Groups C, D, and E, with methadone or buprenorphine. During tests, saline or an increasing dose of drug was administered at the start of each of five to eight successive TO components, and completion of 15 consecutive responses on either lever produced food during each ratio component. Each injection dose increased the cumulative dose by 0.25 or 0.50 common log unit. At least three training sessions were conducted between successive tests. If a subject failed to display criterion performance during a training session, further tests were postponed until at least four sessions of criterion performance occurred. At least one test of cumulative doses of morphine was conducted in each subject. Then subjects were tested for development of tolerance as described below.

Repeated treatment. To examine development of tolerance, each agonist was tested before and after two or more morphine treatment regimens. Different agonists were tested in different groups of rats. Initially, two or more generalization tests were conducted at approximately 1-week intervals. Then training sessions were halted, and a dose of 10 mg/kg morphine was administered once or at 10- to 14-h intervals for periods of one day to two weeks. At the end of each predetermined treatment period, the agonist was retested 12 h after the last injection of morphine. If tolerance was observed, injections of saline were administered at 10- to 14-h intervals for the next five days, and the agonist was tested at 2-day intervals until sensitivity returned to initial levels. Then training resumed. During 2-week treatment regimens, subjects were also tested at the end of the first week of treatment, 12 h after an injection of 10 mg/kg morphine. Subjects in each group were tested for tolerance following one or more treatment regimens (Table 1), with at least three weeks of discrimination training and one morphine generalization test conducted between successive regimens.

Data Analysis

Discriminative performance and response rates were analyzed separately. Discriminative performance, analyzed only if 15 or more responses were emitted during a test trial, is presented as

TABLE 1

ORDER OF CROSS-TOLERANCE TESTS AND INITIAL SENSITIVITY TO MORPHINE (MS)

Test Drug	Subjects and Order of Treatment*	ED_{50} (95% CL) of MS,† in mg/kg Stimulus Control	Rate Suppression
MS			
1 tmt.	Group A (1st, N=6)	1.5 (1.2–1.9)	2.7 (2.4–3.0)
1 day	Group A (2nd, N=5‡)	1.6 (1.2–2.2)	2.8 (2.5–3.1)
3 days	Group A (3rd, N=7‡)	1.2 (0.90–1.5)	2.9 (2.5–3.2)
1–2 weeks	Group B¶ (2nd, N=5)	0.79 (0.68–0.93)	4.1 (2.8–5.9)
Methadone			
3 days	Group C (2nd, N=5§)	1.2 (0.90–1.6)	3.0 (2.5–3.7)
1–2 weeks	Group C¶ (1st, N=6)	0.94 (0.82–1.1)	2.2 (1.9–2.7)
Buprenorphine			
3 days	Group D (1st, N=5)	1.0 (0.94–1.1)	3.2 (2.8–3.6)
1–2 weeks	Group E (1st, N=5)	0.88 (0.75–1.0)	2.9 (2.5–3.4)

*Numbers in parentheses indicate order of tests and number of subjects tested. Each agonist was tested before, during, and after treatment with 10 mg/kg doses of MS.

†Values derived from mean of 1–4 observations in each subject.

‡One subject died before the 1-day treatment. Before the 3-day treatment, one subject stopped responding and was removed, and three subjects were added.

§One subject lost stimulus control before the 3-day treatment.

¶Previously reported (28,29) and included here for comparison with shorter treatment periods. Subjects in group B were tested first during daily treatment with 3.2 mg/kg MS, followed by tests during twice daily treatment with 10 mg/kg MS.

the percentage of responses to the drug-appropriate lever. Rates of responding on either lever are expressed as a percentage of the average rate during saline trials conducted during the 10 sessions that immediately preceded initiation of a repeated treatment regimen. Dose-response functions were constructed by pooling measures of discriminative performance or response rate for all subjects in a group and plotting the resulting mean values as a function of dose. For each treatment regimen, the agonist dose-response functions conducted immediately before initiation of treatment (N = 2 to 5) were pooled to construct a control function. Doses required to evoke 50% drug-appropriate responses or 50% rate suppression (ED_{50} and 95% C.L.) were determined by regression analysis and analysis of variance, with repeated measures where appropriate (23). In order to estimate the magnitude of tolerance at each test, a tolerance ratio and 95% C.L., expressed as ED_{50} after treatment/ED_{50} before treatment, were determined by parallel line assay (13), with significance set at $p<0.05$. For comparisons of changes in sensitivity for individual subjects across treatment regimens, tolerance ratios were calculated by a published microcomputer program (24).

Drugs

Morphine sulfate (obtained from NIDA) and methadone hydrochloride (Sigma) were prepared in physiological saline; buprenorphine hydrochloride (NIDA) was prepared in sterile water. Solutions were prepared to deliver each dose in an injection volume of 0.20 to 0.80 ml. Injections of saline, training doses, and daily treatment doses of morphine were administered in a volume of 0.1 ml per 0.1 kg of body weight. Doses refer to the salts. Injections were administered SC along the dorsal flank.

RESULTS

General Characteristics of Discriminative Performance

Establishment of stimulus control by saline and 3.2 mg/kg morphine required an average ±S.E. of 78±12 sessions in Group A, 72±4 sessions in Group B, 35±3 sessions in Group C, 88±11 sessions in Group D, and 60±3 sessions in Group E. The groups differed slightly in initial sensitivity to stimulus and rate-altering effects of morphine (Table 1), but these differences did not appear to co-vary with the number of sessions required for initial training. The ED_{50} for stimulus control by morphine ranged from 0.79 to 1.6 mg/kg. The ED_{50} for rate suppression ranged from 2.2 to 4.1 mg/kg and did not co-vary systematically with that for stimulus control.

Changes in Sensitivity to Morphine

Sensitivity to discriminative effects of morphine diminished when training was suspended and a dose of 10 mg/kg morphine administered twice daily for periods of one day to two weeks (Fig. 1, left panel, and Table 2). Dose-response functions were parallel across repeated tests, allowing comparisons of changes in ED_{50} as a function of treatment duration. Sensitivity to stimulus effects of morphine diminished as treatment duration increased. One treatment with 10 mg/kg morphine did not alter the ED_{50} for stimulus control, whereas treatment for one or three days increased the ED_{50} 1.7- or 2.4-fold, respectively. As reported previously (28), extending the duration of treatment to one or two weeks increased the ED_{50} 3.2- to 5.3-fold. Longer treatment regimens produced larger changes in sensitivity, with a greater loss of sensitivity after two weeks of treatment than after one or three days of treatment. When treatment ended, sensitivity to stimulus effects of morphine recovered within five days, with slower recovery following longer treatment.

Because some subjects in Group A were exposed to several treatment regimens, comparison of changes in sensitivity within subjects was possible. In general, changes for individual subjects paralleled those for the group (Table 3). One treatment with 10 mg/kg morphine did not decrease sensitivity in any subject, whereas treatment for one or three days decreased sensitivity, with generally larger changes after longer treatment.

During treatment with morphine, response rates often were

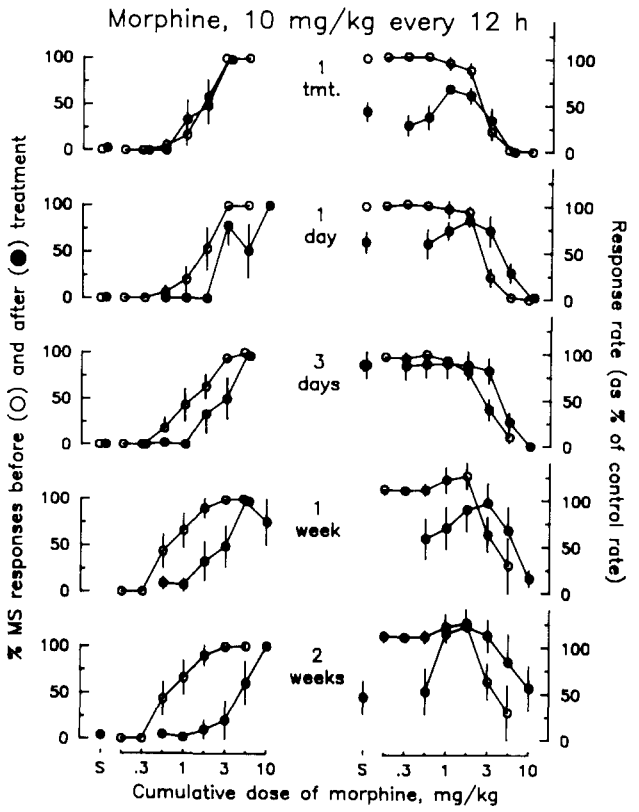

FIG. 1. Dose-response functions for stimulus (left panel) and rate-altering (right panel) effects of morphine in subjects treated for different periods of time with 10 mg/kg morphine, arranged by duration (top to bottom) of repeated treatment. Abscissae: Doses of morphine, log scale. Ordinates, left column: Responses to the morphine-appropriate lever, expressed as a percentage of total trial responses. Ordinates, right column: Response rates, expressed as a percentage of control rates. Group assignments, number of subjects and order of treatments are given in Table 1. Open circles represent control tests conducted before the start of repeated treatment; each point is the mean of 2 to 4 observations in individual subjects before treatment for the period indicated. Closed circles represent tests conducted after treatment for the period indicated; each point is the mean of 1 observation in individual subjects. Vertical bars indicate ±1 S.E. Data in the lower two panels are replotted from Young et al. (28).

suppressed following injection of saline or low doses of morphine (Fig. 1, right panel), although such effects did not co-vary systematically with duration of treatment. Doses of morphine lower than those needed for stimulus control restored rates to control values. The dose needed to suppress rates by more than 50% did not change following one treatment with 10 mg/kg morphine, increased approximately 1.5-fold after one to three days of treatment, and increased more than 2-fold after two weeks of treatment (Table 2). Sensitivity to morphine returned to control levels within three to five days after the end of shorter treatment regimens, but did not fully recover within five days after the 2-week regimen.

Changes in Sensitivity to Other Mu Agonists

Methadone and buprenorphine evoked morphine-like stimulus control, and one or two weeks of treatment with morphine diminished sensitivity to the morphine-like effects of each drug (Table 4). Prior to repeated treatment, methadone evoked morphine-like stimulus effects with an ED_{50} of 0.62 to 0.72 mg/kg. A dose of 1.0 mg/kg evoked full generalization, and higher doses markedly suppressed responding. Repeated treatment with 10 mg/kg morphine for three days produced a small but nonsignificant decrease in sensitivity to methadone, whereas one or two weeks of treatment increased the ED_{50} for methadone 2.2- or 2.5-fold, as reported previously (29). Sensitivity to stimulus effects of methadone returned to control levels within three days after treatment ended. Morphine treatment produced a small loss of sensitivity to rate-suppressing effects of methadone. After two weeks of treatment, response rates were suppressed following injection of saline, but low doses of methadone restored rates to control values, and the ED_{50} for rate-suppression increased only 1.8-fold.

Because some subjects in Group C were exposed to two treatment regimens, comparison of changes in sensitivity within subjects was possible. In general, changes in individual subjects paralleled those for the group (Table 3). In four of five subjects, treatment for one week produced a greater loss of sensitivity than did treatment for three days, but treatment for two weeks generally did not produce a further loss of sensitivity.

Prior to repeated treatment, buprenorphine evoked morphine-like stimulus effects, with an ED_{50} of 0.013 to 0.022 mg/kg. Doses of 0.032 to 0.1 mg/kg evoked full generalization. High doses suppressed response rates, with cumulative doses of 0.1 and 0.3 mg/kg suppressing rates both acutely and up to 24 h after administration. However, such prolonged rate suppression was not observed consistently, nor was it accompanied by either morphine-like stimulus effects or antagonism of stimulus or rate-altering effects of the 3.2 mg/kg training dose of morphine (data not shown). Repeated treatment with morphine for three days did not change the ED_{50} for stimulus control, whereas treatment for one to two weeks increased the ED_{50} 2- to 3-fold (Table 4). During treatment, response rates were suppressed. After three days of treatment, buprenorphine doses of 0.003–1.0 mg/kg did not restore rates. After one or two weeks of treatment, a dose of 0.01 mg/kg restored rates to over 50% of control values, and rates remained approximately 50–70% of control values following cumulative doses of 0.1 to 10 mg/kg. The dose of buprenorphine required for stimulus control returned to initial values within three days after treatment ended, but the dose required for rate suppression remained elevated. However, the 3.2 mg/kg training dose of morphine evoked characteristic stimulus control and rate-altering effects when training was reinitiated (data not shown).

DISCUSSION

Tolerance to discriminative stimulus effects of morphine developed gradually, over a period of days. In rats trained with 3.2 mg/kg morphine, sensitivity to stimulus effects of morphine was not altered by a single treatment with 10 mg/kg morphine [cf. (21)]. Sensitivity progressively decreased as the duration of treatment was extended to one or three days. In agreement with Shannon and Holtzman (22), an approximately 2-fold tolerance to stimulus effects of morphine developed within three days of treatment with twice daily doses of 10 mg/kg morphine. As reported previously, treatment for one or two weeks produced a 3- to 5-fold tolerance (28). Comparisons across groups suggested that greater tolerance developed with longer treatment.

The gradual onset of tolerance produced by repeated injections of morphine parallels that produced by continuous infusions of opioids. In rats trained with 0.04 mg/kg fentanyl, continuously infused fentanyl evokes stimulus control within 8 h

TABLE 2

ED_{50} FOR MORPHINE (MS) BEFORE AND AFTER REPEATED TREATMENT WITH MS

	Stimulus Control				Rate Suppression			
	ED_{50}†	(CL)	Tolerance Ratio	(CL)	ED_{50}†	(CL)	Tolerance Ratio	(CL)
Group A: Treated with 1 injection of MS, 10 mg/kg								
Control	1.5	(1.2–1.9)			2.7	(2.4-3.0)		
After, 12 h	1.5	(1.1–2.1)	1.0	(0.67–1.5)	2.3	(1.8–2.9)	0.92	(0.77–1.1)
Group A: Treated for 1 day with MS, 10 mg/kg ×2								
Control	1.6	(1.2–2.2)			2.8	(2.5–3.1)		
After, 12 h	2.6*	(2.1–3.2)	1.7	(1.2–2.6)	4.4*	(3.6–5.4)	1.7	(1.4–2.0)
3 days	1.9	(1.5–4.0)	1.2	(0.65–2.4)	3.1	(2.7–3.5)	1.1	(0.94–1.3)
5 days	1.5	(1.0–2.2)	0.94	(0.56–1.8)	3.1	(2.6–3.7)	1.1	(0.95–1.3)
Group A: Treated for 3 days with MS, 10 mg/kg ×2								
Control	1.2	(0.90–1.5)			2.9	(2.5–3.2)		
After, 12 h	2.7*	(1.9–3.8)	2.4	(1.5–3.7)	4.7*	(4.0–5.5)	1.6	(1.3–1.9)
3 days	2.2*	(1.4–3.5)	1.8	(1.2–3.0)	5.9*	(5.0–7.0)	2.0	(1.6–2.7)
5 days	2.2	(1.4–3.4)	1.6	(1.0–2.4)	3.4	(3.0–3.8)	1.2	(0.97–1.4)
Group B: Treated for 2 weeks with MS, 10 mg/kg ×2‡								
Control	0.79	(0.70–0.90)			5.8	(4.9–6.7)		
Week 1, 12 h	2.6*	(2.3–2.9)	3.2	(2.1–5.1)	7.1	(6.1–8.3)	1.5	(0.85–2.3)
Week 2, 12 h	4.5*	(4.0–5.1)	5.3	(4.0–7.2)	>10§			
1 day	2.3*	(2.1–2.7)	3.1	(1.9–5.7)	7.0	(6.0–8.1)	1.3	(0.79–2.2)
3 days	1.5*	(1.3–1.7)	1.9	(1.2–3.3)	>5.6			
5 days	0.99	(0.88–1.1)	1.4	(0.84–2.4)	>5.6			

*Significantly different from control value, $p<0.05$.

†Unless noted, test functions were parallel to control function, and tolerance ratios (ED_{50} after treatment/ED_{50} before treatment) were determined by parallel line assays.

‡Previously reported (28,29) and included here for comparison with shorter treatments.

§Response rates were above 50% of control values at all doses tested. The highest dose tested, in mg/kg, is given.

of the onset of infusion, and tolerance to these effects begins to develop within 12 to 48 h (9). The present results also extend a previous report that tolerance to stimulus effects of morphine can appear one day after a single treatment with a high dose of morphine. Witkin and colleagues (25) showed that, in pigeons trained with 1.0 mg/kg morphine, a single dose of 10 mg/kg morphine, given 24 h before testing, increased the dose of morphine required for stimulus control by roughly 3- to 5-fold. In the present experiment, two treatments with 10 mg/kg morphine were needed to increase the dose of morphine required for stimulus control in rats trained with 3.2 mg/kg morphine, suggesting that the ratio between training and treatment doses may control the rate of tolerance development [cf. (28)].

In agreement with previous studies (22,29), repeated treatment with morphine produced cross-tolerance to the μ agonists methadone and buprenorphine. As with morphine, greater tolerance developed with longer treatment. Treatment for three days produced small but nonsignificant changes in sensitivity to both drugs, and treatment for one to two weeks decreased sensitivity by approximately 2-fold. Tolerance to stimulus effects of mor-

TABLE 3

TOLERANCE RATIOS FOR INDIVIDUAL SUBJECTS AFTER TREATMENT FOR DIFFERENT PERIODS*

	Group A: Tests With Morphine				Group C: Tests With Methadone		
	Treatment Duration				Treatment Duration		
Subject	1 Day	1 Day	3 Days	Subject	1 Day	1 Week	2 Weeks†
DD 38	1.0	2.7	2.8	SD 26	1.0	2.3	3.0
DD 49	0.6	1.8	4.4	SD 27	0.6	2.3	2.3
DD 40	1.0	4.2	—‡	SD 28	3.1	2.3	2.3
DD 41	1.0	1.2	1.6	SD 31	2.0	2.3	2.3
DD 43	0.8	1.0	1.8	DD 01	1.4	1.5	3.6

*Tolerance ratios (dose after treatment/dose before treatment) were calculated by a published computer program (24).

†Treatment continued for an additional week after one-week test [from (29)].

‡Subject lost stimulus control before 3-day treatment.

TABLE 4

ED_{50} FOR METHADONE AND BUPRENORPHINE BEFORE AND AFTER REPEATED TREATMENT WITH MORPHINE (MS)

	Stimulus Control				Rate Suppression			
	ED_{50}†	(CL)	Tolerance Ratio	(CL)	ED_{50}†	(CL)	Tolerance Ratio	(CL)
Methadone: Group C, treated for 3 days with MS, 10 mg/kg ×2								
Control	0.62	(0.55–0.69)			1.5	(1.2–1.7)		
After, 12 h	0.91	(0.65–1.3)	1.5	(0.99–2.1)	1.5	(1.3–1.7)	1.0	(0.81–1.2)
3 days	0.71	(0.52–0.99)	1.1	(0.83–1.5)	1.3	(1.1–1.5)	0.86	(0.68–1.1)
7 days	0.81	(0.81–0.81)	1.1	(1.0–1.2)	1.2	(1.0–1.4)	0.85	(0.68–1.1)
Methadone: Group C, treated for 1–2 weeks with MS, 10 mg/kg ×2‡								
Control	0.72	(0.65–0.80)						
Week 1, 12 h	1.7*	(1.1–2.6)	2.2	(1.7–3.2)	1.9	(1.7–2.2)		
Week 2, 12 h	1.8*	(1.5–2.2)	2.5	(2.0–3.0)	2.3	(1.7–3.0)	1.2	(0.77–1.8)
1 day	1.6*	(1.1–2.5)	2.2	(1.6–3.2)	3.2*	(2.1–5.0)	1.8	(1.4–2.5)
3 days	0.77	(0.64–0.92)	1.1	(0.87–1.3)	0.67§	(0.48–0.92)	0.41	(0.29–0.53)
5 days	0.65	(0.49–0.87)	0.91	(0.68–1.2)	2.0	(1.7–2.4)	1.1	(0.87–1.3)
Buprenorphine: Group D, treated for 3 days with MS, 10 mg/kg ×2								
Control	0.022	(0.014–0.033)			0.050	(0.036–0.070)		
After, 12 h	0.029	(0.013–0.065)	1.2	(0.77–2.0)	—¶			
7 days	0.017	(0.007–0.039)	0.92	(0.35–2.3)	0.041	(0.018–0.097)	0.068	(0.28–2.1)
Buprenorphine: Group E, treated for 2 weeks with MS, 10 mg/kg ×2								
Control	0.013	(0.010–0.017)			0.039	(0.025–0.061)		
Week 1, 12 h	0.029*	(0.013–0.065)	2.2	(1.1–4.0)	>10#			
Week 2, 12 h	0.032*	(0.032–0.032)	3.4	(1.7–3.3)	>10			
3 days	0.010	(0.004–0.022)	0.74	(0.30–1.5)	>0.1			

*Significantly different from control value, $p<0.05$.

†Unless noted, test functions were parallel to control function, and tolerance ratios (ED_{50} after treatment/ED_{50} before treatment) were determined by parallel line assays.

‡Previously reported (29) and included here for comparison with shorter treatments.

§Significantly different from control value, but functions were not parallel.

¶Response rates were less than 50% of control values at all doses tested (0.0032–0.32 mg/kg).

#Response rates were above 50% of control values at all doses tested. The highest dose tested, in mg/kg, is given.

phine and buprenorphine generally was similar. In contrast, repeated treatment with morphine produced slightly less tolerance to stimulus effects of methadone than to those of morphine itself. Previous studies have also reported that repeated treatment with morphine can produce less tolerance to the rate-altering and stimulus effects of both *l*- and *dl*-methadone than to similar effects of morphine [(6, 18, 22); but see (17)].

Tolerance to stimulus effects of morphine and methadone generally was accompanied by comparable tolerance to their rate-altering effects. In contrast, much greater tolerance developed to rate-altering effects of buprenorphine than to its morphine-like stimulus effects. After two weeks of treatment with morphine, buprenorphine doses as high as 10 mg/kg did not markedly suppress response rates. A similar loss of maximal effect has been reported for analgesic effects of buprenorphine in rats treated daily with 20 mg/kg morphine (7) and for rate-decreasing effects of buprenorphine under FR schedules of food reinforcement in rats treated daily with 40 mg/kg morphine (20). Sensitivity to rate-altering effects of buprenorphine also appeared to recover more slowly after termination of treatment than did sensitivity to its stimulus effects. It is unlikely that such effects were due to prolonged actions of buprenorphine itself, because the training dose of morphine evoked characteristic stimulus control and rate-altering effects when training was reinitiated. The lesser tolerance and faster recovery observed for stimulus effects of buprenorphine may indicate that such effects are achieved with lower receptor occupancy than are analgesic or rate-suppressing effects of buprenorphine [cf. (2,3)].

Sensitivity to stimulus and rate-altering effects of morphine, methadone, and buprenorphine generally recovered after termination of repeated treatment, with faster recovery after shorter treatments that produced lower tolerance. The present experiments, however, may overestimate the time required for recovery of stimulus and rate-altering effects of morphine, inasmuch as repeated testing may have reinitiated tolerance after treatment ended. Nonetheless, since any reinitiation of tolerance would have occurred in all groups tested with a particular agonist, the present data suggest that rate of recovery was faster after shorter treatment periods. In all experiments, discrimination training did not resume until sensitivity to stimulus effects of morphine returned to initial values. Such recovery of sensitivity suggests that development of tolerance to stimulus effects of morphine reflected a pharmacodynamic process rather than establishment of control by a higher training dose [cf. (4)], inasmuch as the effects of conditioning a higher training dose would be expected to persist after the end of treatment.

Onset and magnitude of tolerance to opioids are determined jointly by the treatment dose and the interval between doses [cf. (5,15)]. The present study examined only a dose of 10 mg/kg given at 12-h intervals. Changes in the daily dose of morphine produce corresponding changes in magnitude of tolerance to stimulus effects of morphine (28), and it is likely that changes in the dose or frequency of treatment would produce similar changes in onset of tolerance. However, the gradual onset and offset of tolerance to stimulus effects of morphine seen in the present experiments parallel the onset and offset of tolerance to

certain other μ opioid effects. For example, experiments by Fernandes and colleagues (10–12) have shown that magnitude of tolerance to several analgesic, motor, and physiologic effects of morphine initially increases as treatment duration is increased from 5 to 10 days, plateaus as treatment duration is extended for an additional 10 days, and declines over time after treatment ends. Under other conditions, tolerance to behavioral and analgesic effects of opioids can continue to increase as the duration of treatment is extended beyond one or two weeks [e.g., (1,14)]. It remains to be determined if changes in treatment regimens will also amplify tolerance to discriminative stimulus effects of morphine.

ACKNOWLEDGEMENTS

C. Burke, C. M. Laus and D. S. Shaw provided excellent technical assistance.

REFERENCES

1. Adams, J. U.; Holtzman, S. G. Tolerance and dependence after continuous morphine infusion from osmotic pumps measured by operant responding in rats. Psychopharmacology (Berlin) 100:451–458; 1990.
2. Blasig, J.; Meyer, G.; Hollt, V.; Hengstenberg, J.; Dum, J.; Herz, A. Non-competitive nature of the antagonistic mechanism responsible for tolerance development to opiate-induced analgesia. Neuropharmacology 18:473–481; 1979.
3. Chavkin, C.; Goldstein, A. Opioid receptor reserve in normal and morphine-tolerance guinea pig ileum myenteric plexus. Proc. Natl. Acad. Sci. USA 81:7253–7257; 1984.
4. Colpaert, F. C. Discriminative stimulus properties of narcotic analgesic drugs. Pharmacol. Biochem. Behav. 9:863–887; 1978.
5. Cox, B. M.; Ginsburg, M.; Osman, O. H. Acute tolerance to narcotic analgesic drugs in rats. Br. J. Pharmacol. Chemother. 33:245–256; 1968.
6. Craft, R. M.; Picker, M. J.; Dykstra, L. A. Differential cross-tolerance to opioid agonists in morphine-tolerant pigeons responding under a schedule of food presentation. J. Pharmacol. Exp. Ther. 249:386–393; 1989.
7. Dum, J.; Blasig, J.; Herz, A. Buprenorphine: Demonstration of physical dependence liability. Eur. J. Pharmacol. 70:293–300; 1981.
8. Emmett-Oglesby, M. W.; Shippenberg, T. S.; Herz, A. Tolerance and cross-tolerance to the discriminative stimulus properties of fentanyl and morphine. J. Pharmacol. Exp. Ther. 245:17–23; 1988.
9. Emmett-Oglesby, M. W.; Shippenberg, T. S.; Herz, A. Fentanyl and morphine discrimination in rats continuously infused with fentanyl. Behav. Pharmacol. 1:3–11; 1989.
10. Fernandes, M.; Kluwe, S.; Coper, H. Quantitative assessment of tolerance to and dependence on morphine in mice. Naunyn Schmiedebergs Arch. Pharmacol. 297:53–60; 1977.
11. Fernandes, M.; Kluwe, S.; Coper, H. The development of tolerance to morphine in the rat. Psychopharmacology (Berlin) 54:197–201; 1977.
12. Fernandes, M.; Kluwe, S.; Coper, H. Development and loss of tolerance to morphine in the rat. Psychopharmacology (Berlin) 78:234–238; 1982.
13. Finney, D. J. Statistical methods in biological assay, 2nd edition. New York: Hafner; 1964.
14. France, C. P.; Woods, J. H. Effects of morphine, naltrexone, and dextrorphan in untreated and morphine-treated pigeons. Psychopharmacology (Berlin) 85:377–382; 1985.
15. Goldstein, A.; Sheehan, P. Tolerance to opioid narcotics. I. Tolerance to the "running fit" caused by levorphanol in the mouse. J. Pharmacol. Exp. Ther. 169:175–184; 1969.
16. Kapitsopoulos, G.; Young, A. M. Buprenorphine-induced tolerance to discriminative stimulus effects of morphine and buprenorphine. Soc. Neurosci. Abstr. 16:929; 1990.
17. Leander, J. D.; McMillan, D. E.; Harris, L. S. Effects of narcotic agonists and antagonists on schedule-induced water and morphine ingestion. J. Pharmacol. Exp. Ther. 195:271–278; 1975.
18. McMillan, D. E.; McGivney, W. T.; Hardwick, W. C. Effects of drugs on behavior in rats maintained on morphine, methadone or pentobarbital. J. Pharmacol. Exp. Ther. 215:9–14; 1980.
19. Miksic, S.; Lal, H. Tolerance to morphine-produced discriminative stimuli and analgesia. Psychopharmacology (Berlin) 54:217–221; 1977.
20. Negus, S. S.; Picker, M. J.; Dykstra, L. A. Kappa antagonist properties of buprenorphine in non-tolerant and morphine-tolerant rats. Psychopharmacology (Berlin) 98:141–143; 1989.
21. Sannerud, C. A.; Young, A. M. Environmental modification of tolerance to morphine discriminative stimulus properties in rats. Psychopharmacology (Berlin) 93:59–68; 1987.
22. Shannon, H. E.; Holtzman, S. G. Evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 198:54–65; 1976.
23. Snedecor, G. W.; Cochran, W. G. Statistical methods, 6th edition. Ames, IA: Iowa State University Press; 1967.
24. Tallarida, R. J.; Murray R. B. Manual of pharmacologic calculations with computer programs, second edition. New York: Springer-Verlag; 1987.
25. Witkin, J. M.; Dykstra, L. A.; Carter, R. B. Acute tolerance to the discriminative stimulus properties of morphine. Pharmacol. Biochem. Behav. 17:223–228; 1982.
26. Wood, D. M.; Emmett-Oglesby, M. Characteristics of tolerance, recovery from tolerance and cross-tolerance for cocaine used as a discriminative stimulus. J. Pharmacol. Exp. Ther. 237:120–125; 1986.
27. Young, A. M.; Sannerud, C. A. Tolerance to drug discriminative stimuli. In: Goudie, A. J; Emmett-Oglesby, M. W., eds. Psychoactive drugs. Clifton, NJ: Humana Press; 1989:221–278.
28. Young, A. M.; Sannerud, C. A.; Steigerwald, E. S.; Doty, M. D.; Lipinski, W. J.; Tetrick, L. Tolerance to morphine stimulus control: Role of morphine maintenance dose. Psychopharmacology (Berlin) 102:59–67; 1990.
29. Young, A. M.; Kapitsopoulos, G.; Makhay, M. Tolerance to morphine-like stimulus effects of mu opioid agonists. J. Pharmacol. Exp. Ther: 257:795–805; 1991.