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Opioid receptor subtype-specific cross-tolerance to the effects of morphine on schedule-controlled behavior in mice

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Abstract. Key-press responding of mice was maintained under a fixed-ratio (FR) 30-response schedule of food presentation. Successive 3-min periods during which the experimental chamber was illuminated and the schedule was in effect were preceded by 10-min time-out (TO) periods during which all lights were out and responses had no scheduled consequences. Intraperitoneal (IP) injections of saline or of cumulative doses of drugs were given at the start of each TO period. Successive saline injections had little or no effect on response rates, whereas the μ-opioid agonists morphine (0.1–10.0 mg/kg) and levorphanol (0.1–3.0 mg/kg), the κ-opioid agonist ethylketazocine (0.03–3.0 mg/kg), the mixed μ-/δ-opioid agonist metkephamid (0.1–10.0 mg/kg), and the nonopioid dissociative anesthetic ketamine (1.0–100.0 mg/kg) generally produced dose-related decreases in response rates. Following chronic administration of morphine (100.0 mg/kg/6 h), tolerance developed to the effects of morphine on rates of responding. In addition, a comparable degree of cross-tolerance developed to the effects of levorphanol and metkephamid. On the other hand, there was no evidence of cross-tolerance to the effects of ethylketazocine or ketamine. These results are consistent with the evidence suggesting that different opioid agonists exert their behavioral effects through distinct classes of opioid receptors.

Key words: Mice – Schedule-controlled behavior – Tolerance – Morphine – Ketamine – Ethylketazocine – Metkephamid – Levorphanol – Cross-tolerance

One characteristic observation with repeated administration of opioids is the development of tolerance to many of their effects. The tolerance that develops to opioid effects may derive from a specific decrease in the density of opioid receptors (e.g., Davis et al. 1979; however, see Law et al. 1984). Thus, the display of cross-tolerance between different opioids might indicate commonalities in their mechanisms of action (e.g., that they are agonists at the same opioid receptor type). For example, Schulz and Wuster (1981) chronically treated mice with either the μ agonist sufentanyl, several κ agonists, or the δ agonist [D-Ala^2,D-Leu^5]enkephalin (DADL), and subsequently studied the response to opioids of the electrically-driven vas deferens of these subjects. Subjects treated with sufentanyl developed tolerance to the effects of the drug; however, cross-tolerance was not conferred to the κ or δ agonists. In general, similar effects were observed with tolerance to the other compounds; though cross tolerance to the κ agonists did not confer to other κ agonists the same degree of cross tolerance. The authors suggested that subtypes of μ and κ receptors may account for the different degrees of cross-tolerance conferred. Selective tolerance between μ and δ agonists has also been observed in vivo for some behavioral effects of sufentanyl and DADL (Schulz et al. 1981).

The purpose of the present study was to evaluate the specificity of tolerance to effects of morphine on schedule-controlled behavior in mice. To this end, tolerance was produced to the effects of morphine, and cross-tolerance was assessed to other opioid agonists and to the non-opioid agonist ketamine. Opioid agonists studied in addition to morphine were the μ agonist levorphanol, the κ agonist ethylketazocine and the μ/δ agonist metkephamid. Metkephamid was of particular interest, as there are inconsistencies with regard to whether morphine confers cross-tolerance to this drug. For example, initial studies of analgesic effects in mice indicated no cross-tolerance conferred to metkephamid with morphine treatment (Frederickson et al. 1981; Hynes et al. 1982). Similarly, Locke and Holtzman (1986) reported no cross-tolerance to metkephamid with morphine treatment on locomotor activity. Others, however, have reported cross-tolerance to the effects of morphine and metkephamid on operant behavior in pigeons (Leander and Wood 1982) and analgesic effects in mice (Leander 1987). Thus, determinants of whether cross-tolerance is observed may be related to the effect being studied. Further, with mice there is inconsistent data with regard to whether cross-tolerance to metkephamid is conferred by morphine treatment.

Methods

Subjects. Three adult male Swiss-Webster mice originally weighing 30.0–37.5 g were used. Subjects were housed indi-

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vidually and had unrestricted access to water in their home cages. Body weights were maintained at 80% of the unrestricted feeding weights by adjusting the daily rations of food pellets (P.J. Noyes, Lancaster, NH) that supplemented milk obtained during experimental sessions. The daily or weekend ration of pellets was given to subjects after sessions. The subjects were experimentally naive at the start of the experiment.

Apparatus. During experimental sessions the mice were placed in a chamber described previously (Katz 1984) which was placed in a ventilated, sound-attenuating aluminum shell. A small aluminum-floored corridor at the front of the chamber had a small-rodent response paddle (BRS/LVE, Beltsville, MD, #RLP-003) at one end. Depression of the paddle with a force exceeding 2 g (0.02 N) produced a click of a relay mounted outside the chamber and was recorded as a response. Overhead illumination, provided by a white lamp (7.5 W, 120 V, a.c.) served as a visual stimulus. White noise, provided by a speaker mounted on the back wall of the chamber, served as an auditory stimulus. A solenoid-operated dipper (R. Gebrands Co., Arlington, MA, modified Model B-LH) delivered 0.025 ml of evaporated milk through a hole in the floor of the corridor in front of the response paddle.

Procedure. Mice were trained to depress the response paddle during experimental sessions that were conducted five days per week. Sessions began with a 10-min timeout (TO) period during which the white noise and the overhead illumination were off and responses had no scheduled consequences. The TO was followed by a 3-min period during which the white noise and overhead illumination were on and responses produced milk according to a fixed-ratio (FR) schedule. Under the FR schedule each 30th response produced access to evaporated milk via the dipper that was accessible for 3 s. Sessions were comprised of five TO periods, each followed by a 3-min period of FR reinforcement.

Once performances were stable on a day-to-day basis (no increasing or decreasing trends in response rates), subjects were adapted to within-session saline injections. During selected sessions two or three times per week, saline injections were administered IP at the start of each TO period. When injection and handling of the subject no longer disrupted the behavioral performance, effects of drugs were assessed. Before selected sessions, and no more frequently than once per week with opioids or once every 3 days for ketamine (typically Tuesdays and Fridays), drugs were administered in increasing cumulative doses at the start of each timeout period. Each successive injection was of an amount of drug that incremented the total dose injected within that session by one-half log unit.

Once dose-effect curves for each of the drugs were determined, subjects were rendered tolerant to effects of morphine by administering the drug every 6 h. During the 1st week of chronic morphine, injections of 30.0 mg/kg were given at 01:00, 07:00, 13:00 and 19:00 hours. Following the 1st week, the dose was increased to 100.0 mg/kg/6 h. During the morphine treatment regimen, experimental sessions were conducted from 2 to 4 h following the 07:00 hour morphine injection, the time of day at which sessions were conducted before chronic morphine treatment. Subjects were readapted to the injection procedure with saline injections and were then tested again with the various drugs studied prior to the induction of morphine tolerance.

Analysis of results. Response rates were calculated by dividing total responses by elapsed time during the fixed-ratio component for individual subjects. Response rates were transformed to a percentage of rates occurring during the corresponding component during vehicle control sessions. Percentage of control data on the linear portions of the dose-effect curves were analyzed by standard parallel line bioassay techniques (Finney 1964) to obtain potency estimates. Analysis of variance and linear regression techniques (Snedecor and Cochran 1967) were used to determine ED_{50} values (the dose causing a decrease in response rates to 50% of the control rate) and 95% confidence limits.

Drugs. Morphine sulfate (NIDA), ethylketazocine methanesulfonate (Sterling-Winthrop, Rensselaer, NY), levorphanol tartrate (Hoffmann-La Roche, Nutley, NJ), ketamine hydrochloride (Warner-Lambert, Ann Arbor, MI) and metkephamid (Try-D-Ala-Gly-Phe-N(Me)Met-amide; Eli Lilly, Indianapolis, IN) were dissolved in sterile saline (0.9% NaCl) or distilled water for injection. All drugs were administered intraperitoneally (IP). Doses are expressed as the above forms of the drugs in mg per kg body weight of the subject.

Results

In the absence of injections, responding of mice under the FR 30 schedule was similar to that typically seen with this type of schedule (Ferster and Skinner 1957). At the onset of each component there was a brief pause that was followed by an abrupt transition to responding at a high rate that was maintained until milk presentation. Little or no responding occurred during the TO periods. When saline injections were administered prior to each component of the daily sessions, the rates of responding were similar across the successive components before and during chronic morphine administration (Fig. 1, panel *A*). Repeated measures analysis of variance of data collected after saline injections showed no significant difference in response rates with repeated injections [$F(4,16 = 1.23$], before or during chronic morphine [$F(4,16 = 1.00$], or an interaction [$F(4,16 = 0.343$].

Before its chronic administration, morphine produced dose-related decreases in rates of responding (Fig. 1, panel *B*, filled points). The ED_{50} value was 2.9 mg/kg (Table 1), and responding was virtually eliminated at 10.0 mg/kg. After chronic administration of morphine, the drug continued to produce dose-related decreases in response rates (Fig. 1, panel *B*, unfilled points), but the dose-effect curve was shifted approximately one log unit to the right (the relative potency ratio for morphine was 0.11, Table 1). The ED_{50} value was increased to 27.5 mg/kg (Table 1), and responding was not eliminated up to a dose of 100.0 mg/kg.

Both levorphanol and metkephamid produced patterns of effects similar to that of morphine (Fig. 1, panels *C* and *D*). Dose-related decreases in response rates were observed with both drugs, prior to as well as following chronic morphine treatment. The ED_{50} value of levorphanol was increased from 1.3 to 14.2. For both drugs, the dose-effect curves were shifted approximately 1 log unit to the right as a consequence of chronic morphine administration (the

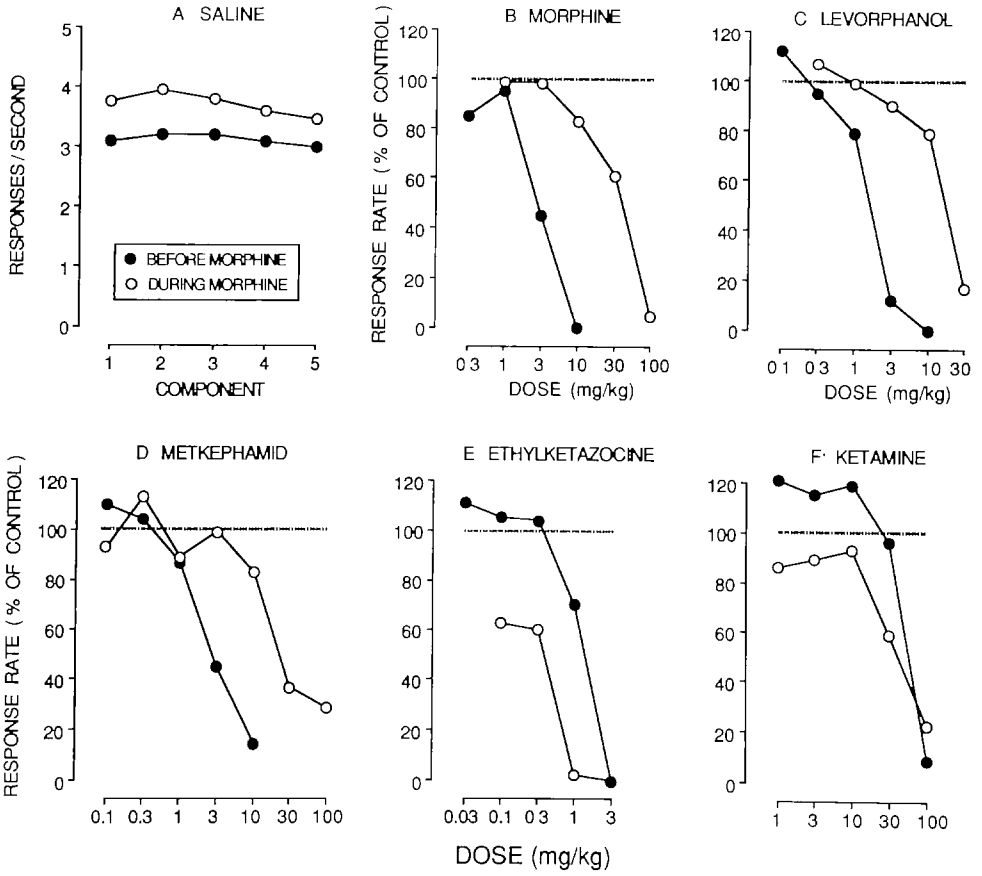

Fig. 1. Effects of saline or cumulative doses of drugs on average rates of responding of mice studied under the FR 30 TO 10-min schedule of milk presentation, prior to and following the development of morphine tolerance. *Upper left panel ordinates*. response rate in responses per s. *Upper left panel abscissae:* Successive components. *Other ordinates:* response rates as a percentage of control (saline) rates. *Other abscissae:* cumulative doses of drugs in mg/kg on logarithmic scales. For each dose of drug, response rates are expressed as a percentage of the average control rates in the corresponding component; for the two highest doses of metkephamid, the average response rates over the entire sessions were used as the control, since a corresponding rate for saline was not available. *Filled points* show the effects of saline injections or drugs prior to chronic morphine treatment; *unfilled points* show effects after chronic morphine treatment

Table 1. Summary of effective doses and relative potencies of compounds before and during the repeated administration of morphine

Drug	ED_{50} Value		
	Before	During	Relative potency
Morphine	2.9 (1.6–5.2)	27.5 (15.6–48.3)	0.11 (0.05–0.21)
Ethylketazocine	1.7 (0.5–2.5)	0.5 (0.4–0.7)	2.10 (0.83–6.49)
Levorphanol	1.3 (0.9–1.9)	14.2 (5.0–40.6)	0.11 (0.05–0.25)
Metkephamid	2.8 (1.6–4.8)	NS	0.10 (0.02–0.43)
Ketamine	57.6 (10.0–331.6)	NS	1.38 (0.32–17.92)

Values for ED_{50} are expressed in mg/kg for data collected before and during the repeated administration of morphine (100 mg/kg/6 h). The values for relative potency represent the dose of the drug, in mg/kg, "before" treatment, equal to 1 mg/kg of the drug "during" treatment. All numbers within parentheses are the 95% confidence limits. Invalid bioassays (NS) were caused by a lack of significance in the linear regression analysis

relative potency ratios for levorphanol and metkephamid were 0.11 and 0.10, respectively, Table 1).

As with the other opioids, ethylketazocine generally produced dose-related decreases in response rates before chronic administration of morphine (Fig. 1, panel *E*, filled points) with an ED_{50} value of 1.7 mg/kg (Table 1). In contrast to the results observed with the other agonists, the dose-effect curve for the effects of ethylketazocine was not shifted to the right with chronic morphine treatment (Fig. 1, panel *E*, unfilled points). Rather, the ED_{50} value was decreased to 0.5 mg/kg and the relative potency estimate was 2.1 (Table 1).

Ketamine produced small increases in response rates at the lesser doses (1.0–10.0 mg/kg), and dose-related decreases in response rates at greater doses (30.0–100.0 mg/kg) prior to chronic morphine treatment (Fig. 1, panel *F*, filled points). After chronic administration of morphine, the lesser doses of ketamine no longer increased response rates, and the doses of ketamine that decreased rates of responding were the same doses that had done so prior to chronic morphine treatment (Fig. 1, panel *F*, unfilled points). The ED_{50} value for the linear portion of the dose-effect curve before chronic treatment was 57.6 mg/kg, with

a relative potency ratio of 1.38, indicating no significant change in potency due to morphine treatment.

Discussion

Key-press responding of mice was maintained by milk reinforcement under an FR 30 schedule in which successive components of the session were preceded by injections of saline or drugs. The multiple saline injections had little or no effect on response rates across the session, and, as has been found previously (e.g., Young 1986; Solomon et al. 1987), the effects of cumulative doses were similar to the effects of single doses. Further, the effects of each of the drugs studied were similar to effects previously reported for these compounds (e.g., McMillan et al. 1970; Thompson et al. 1970; Solomon et al. 1982). These results indicate that the procedure is appropriate for evaluation of the effects of cumulative doses of drugs.

Following chronic treatment with morphine, cross-tolerance was found to have developed to the μ-opioid agonist levorphanol. The degree of shift in the dose-response function for levorphanol was comparable to that seen with morphine, which agrees with earlier results obtained with levorphanol or with the μ-opioid agonist levomethorphan in pigeons and monkeys (Woods and Carney 1978). This result suggests that the display of cross-tolerance to effects of opioids on operant behavior may be dependent on commonalities in their mechanisms of action (e.g., that they act at the same opioid receptor subtype). However, a similar degree of cross-tolerance was seen between morphine and the mixed μ-/δ-opioid agonist metkephamid. In a previous report of the effects of metkephamid on operant behavior in pigeons (Leander and Wood 1982), a more limited degree of cross-tolerance between metkephamid and the μ-opioid agonist methadone was found, and in other studies that examined behavioral or other effects (e.g., antinociception) of these agents, an absence of cross-tolerance between morphine and metkephamid was seen (e.g., Locke and Holtzman 1986; Hynes and Fredrickson 1982). Thus, while metkephamid is considered to possess equal affinity with morphine for the μ-opioid receptor and a greater affinity than morphine for the δ-opioid receptor (Burkhardt et al. 1981), the present finding of cross-tolerance between morphine and metkephamid, along with the report of Leander and Wood (1982), suggest that μ-opioid receptors may be the primary site of action for the effects of metkephamid on operant behavior. In contrast, its action at δ-opioid receptors may be more important for its analgesic effects (Burkhardt et al. 1982; however see Leander 1987).

The absence of morphine-induced cross-tolerance to ethylketazocine is consistent with the theory that these agents act at distinct subtypes of opioid receptors. The possible development of sensitization to the effects of ethylketazocine, however, is more difficult to account for. One group of agents that show sensitization to their effects following chronic treatment with morphine are the opioid antagonists (e.g., Valentino et al. 1983). However, an opioid-antagonist action of ethylketazocine is not likely, since this drug fails to precipitate opioid withdrawal in morphine-dependent rhesus monkeys (e.g., Swain and Seevers 1974; see also Woods et al. 1979), and chronic administration of ethylketazocine itself also produces an increased sensitivity to the behavioral effects of opioid antagonists, although to a lesser degree than does morphine (e.g., Tepper and Woods 1978).

The dose-response function for the response-rate decreasing effects of the non-opioid dissociative anesthetic ketamine was generally unaffected by chronic morphine treatment. Ketamine and its structural analog phencyclidine share many pharmacological effects with the σ-opioid agonist N-allylnormetazocine (e.g., Solomon et al. 1981). The present observation of a lack of cross-tolerance between morphine and ketamine suggests that ketamine, and perhaps other σ-opioid agonists as well, do not share important mechanisms of action with μ-opioids, such as morphine.

In conclusion, the results of this study demonstrate that the development of tolerance to morphine is accompanied by a selective pattern of cross-tolerance to other opioid agonists. A substantial degree of cross-tolerance was found between morphine and agonists that also act at μ-opioid receptors, either exclusively (e.g., levorphanol) or in part (e.g., metkephamid). In contrast, there was no cross-tolerance to the κ-opioid agonist ethylketazocine, nor to the dissociative anesthetic ketamine. These results are consistent with the theory that action at a number of receptor subtypes can account for the variety of effects produced by different opioid agonists, and suggest that studies on cross-tolerance may be useful to classify opioids with regard to their mechanisms of action on operant behavior.

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References

Burkhardt C, Fredrickson RCA, Pasternak GW (1982) Metkephamid (Tyr-D-Ala-Gly-Phe-N(Me)-NH_2), a potent opioid peptide: Receptor binding and analgesic properties. Peptides 3:869–871

Davis ME, Akera T, Brody TM (1979) Reduction of opiate binding to brainstem slices associated with the development of tolerance to morphine in rats. J Pharmacol Exp Ther 211:112–119

Ferster CB, Skinner BF (1957) Schedules of reinforcement. Appleton-Century-Crofts, New York

Finney DJ (1964) Statistical methods in biological assay, 2nd ed. Hafner, New York

Frederickson RCA, Smithwick EL, Shuman R, Bemis KG (1981) Metkephamid, a systemically active analog of methionine enkephalin with potent opioid δ-receptor activity. Science 211:603–605

Hynes MD, Fredrickson RCA (1982) Cross-tolerance studies distinguish morphine- and metkephamid-induced analgesia. Life Sci 31:1201–1204

Katz JL (1984) Effects of clonidine and some α-adrenergic antagonists alone and in combination on schedule-controlled behavior in pigeons and mice. Psychopharmacology 83:38–43

Katz JL, Solomon RE, Goodrich JE (1982) Morphine tolerance: cross tolerance and lack of cross-tolerance to different narcotic agonists. Pharmacologist 24:115 (abstract)

Law P-Y, Griffin MT, Loh HH (1984) Mechanisms of multiple cellular adaptation processes in clonal cell lines during chronic opiate treatment. In: Mechanisms of tolerance and dependence, NIDA Research Monograph No. 54, US Government Printing Office, Washington DC, pp 119–135

Leander JD (1987) Cross-tolerance to metkephamid (LY127623) produced by morphine solution ingestion by mice. Alcohol Drug Res 7:321–325

Leander JD, Wood CR (1982) Metkephamid effects on operant behavior. Peptides 3:771–773

Locke KW, Holtzman SG (1986) Behavioral effects of opioid peptides selective for mu or delta receptors. II. Locomotor activity in non-dependent and morphine-dependent rats. J Pharmacol Exp Ther 238:997–1003

McMillan DE, Wolf PS, Carchman RA (1970) Antagonism of the behavioral effects of morphine and methadone by narcotic antagonists in the pigeon. J Pharmacol Exp Ther 175:443–458

Schulz R, Wuster M (1981) Are there subtypes (isoreceptors) of multiple opiate receptors in the mouse vas deferens? Eur J Pharmacol 76:61–66

Schulz R, Wuster M, Herz A (1981) Differentiation of opiate receptors in the brain by the selective development of tolerance. Pharmacol Biochem Behav 14:75–79

Snedecor GW, Cochran WG (1967) Statistical methods, 6th ed. Iowa State University Press, Ames, Iowa, pp 135–171

Solomon RE, Herling S, Woods JH (1981) Ketamine-like discriminative characteristics of the stereoisomers of metazocine, cyclazocine and SKF-10,047 in rhesus monkeys. In: Advances in endogenous and exogenous opioids. Kodansha, Tokyo, pp 484–486

Solomon RE, Herling S, Domino EF, Woods JH (1982) Discriminative stimulus effects of N-substituted analogs of phencyclidine in rhesus monkeys. Neuropharmacology 21:1329–1336

Solomon RE, Wasserman EA, Gebhart GF (1987) Tolerance to antinociceptive effects of morphine without tolerance to its effects on schedule-controlled behavior. Psychopharmacology 92:327–333

Swain HH, Seevers MH (1974) Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. In: Proceedings of the Committee on Problems of Drug Dependence. NAS-NRC Washington, DC, Addendum

Tepper P, Woods JH (1978) Changes in locomotor activity and naloxone-induced jumping in mice produced by WIN 35,197-2 (ethylketazocine) and morphine. Psychopharmacology 58:125–129

Thompson T, Trombley J, Luke D, Lott D (1970) Effects of morphine on behavior maintained by four simple food-reinforcement schedules. Psychopharmacologia 17:182–192

Valentino RJ, Herling S, Woods JH (1983) Discriminative stimulus effects of naltrexone in narcotic-naive and morphine-treated pigeons. J Pharmacol Exp Ther 224:307–313

Woods JH, Carney J (1978) Narcotic tolerance and operant behavior. In: Krasnegor NA (ed) Behavioral tolerance: research and treatment implications. NIDA Research Monograph Series, vol 19, US Government Printing Office, Washington, DC, pp 54–66

Woods JH, Smith CB, Medzihradsky F, Swain HH (1979) Preclinical testing of analgesic drugs. In: Beers RF Jr, Bassett EG (eds) Mechanisms of pain and analgesic compounds. Raven Press, New York, pp 429–445

Young AM (1986) Effects of acute morphine pretreatment on the rate-decreasing and antagonist activity of naloxone. Psychopharmacology 88:201–208

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