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Ketamine- and Morphine-Induced Analgesia and Catalepsy. I. Tolerance, Cross-Tolerance, Potentiation, Residual Morphine Levels and Naloxone Action in the Rat[1]

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ABSTRACT

The effects of ketamine and morphine on pain perception and catalepsy were compared in rats. Analgesia, as measured by the latency to withdrawal of the tail from a 55°C water bath (tail-flick latency difference, TFLD), was produced by both ketamine and morphine, but at widely different doses, and in each case the effect was reversed by naloxone. Catalepsy, measured by the duration of loss of righting reflex (DLRR) in catatonic animals, was induced by larger doses of both ketamine and morphine and in each case was reduced by a larger dose of naloxone. DLRR and TFLD tolerance developed rapidly and with a similar time course after daily doses of ketamine or morphine. Rats tolerant to the DLRR effect of ketamine showed cross-tolerance to morphine. Rats tolerant to the DLRR effect of morphine did not show cross-tolerance to ketamine when administered the following day; instead, these rats showed potentiation of the ketamine-induced DLRR. The degree of potentiation noted 24 hr after a single or multiple daily doses of 45 mg/kg of morphine is the same as that seen when 2 mg/kg of morphine is given simultaneously with ketamine. The residual brain level of morphine 24 hr after 45 mg/kg is similar to the level 1 hr after a 2-mg/kg dose. The augmented ketamine response in morphine-tolerant rats is postulated to be a result of residual morphine still present in the brain 24 hr after the last DLRR-inducing dose of morphine. The development of tolerance, cross-tolerance and potentiation between ketamine and morphine, as well as the reversal of the TFLD and DLRR actions by the opioid antagonist naloxone, suggest a commonality of one or more sites of action of these agents.

In a series of studies, Berkowitz *et al.* (1976, 1977) demonstrated that a low dose of nitrous oxide induced analgesia; the analgesia was reversed by the opioid antagonist naloxone; tolerance developed to daily nitrous oxide and there was cross-tolerance with morphine. Based on these findings, Berkowitz *et al.* (1976) proposed that there could be a similar mechanism of action for both morphine and nitrous oxide. The proposed similarity of CNS activity between nitrous oxide and opioids suggested that other agents could likewise interact with the opioids. For example, the anesthetic and hallucinogenic agents morphine (Nakamura and Winters, 1973), nitrous oxide, γ-hydroxybutyrate, ketamine, phencyclidine, LSD-25, mescaline and ethrane (Winters, 1975), induce a stereotypic continuum of states of CNS excitation (Winters *et al.*, 1972; Winters, 1976, 1982) characterized by a dose/time related progression from sedation (Winters and Kott, 1979), to activation, hallucinosis, catatonia, catalepsy and seizures (Winters *et al.*, 1972). A preliminary study (Lakin and Winters, 1979) in our laboratory demonstrated that the ketamine-induced analgesia, catatonia and catalepsy were all decreased by naloxone. The antagonism of ketamine-induced analgesia by naloxone has been confirmed by De Simoni *et al.* (1983) but not the prevention of ketamine-induced loss of righting reflex (their data not shown).

The recognition that ketamine as well as nitrous oxide induced naloxone-reversible analgesia was of interest as neither is considered an opioid. The relationship of the actions of these two agents to those of the opioids suggested the present studies. In addition, discrepancies between our previous data on loss of righting reflex and the findings of others suggested a necessity for investigations which were designed to compare the dose-response relationships of ketamine and morphine with respect to the induction of analgesia, catatonia and catalepsy. Comparisons of the induction of tolerance, cross-tolerance, naloxone interactions and morphine levels in blood and brain also were made.

Methods

Animals. Female Sprague-Dawley rats weighing 200 to 300 g were housed two per cage and allowed water and food *ad libitum*. Cages were

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ABBREVIATIONS: CNS, central nervous system; TFLD, tail-flick latency difference; DLRR, duration of loss of righting reflex.

placed in a temperature-controlled room and arranged so that each cage received the same level of fluorescent illumination (Vita-lite). The photoperiod was 12 hr light on and 12 hr light off, with the light period from 6:00 A.M. until 6:00 P.M. The rats were acclimated at least 7 days before being used in these studies. To avoid the previously reported seasonal variations in ketamine response (Winters *et al.*, 1986), the majority of experiments were conducted in December through March.

Drugs. The following drugs were used: ketamine (Ketoject, Bristol Laboratories, La Mirada, CA); morphine sulfate (Mallinckrodt, Inc., Paris, KY); and naloxone (Narcan, Endo Laboratories, Garden City, NY). The doses of all drugs were calculated as the base expressed as milligrams per kilogram. Each drug was dissolved in 0.9% saline and administered by the i.p. route.

Test Procedures

All tests were performed 4 to 8 hr after the onset of the light period. The dose-response relationships are reported in three dose ranges; the range which induces analgesia without catatonia, the dose range for catatonia and the dose range which induces catalepsy.

Analgesia. TFLD. Analgesia was defined as a loss of sensibility to noxious stimuli without a loss of conscious awareness of the environment or the loss of ability to respond. Increasing doses producing low levels of analgesia were not accompanied by detectable changes in gross motor activity. At the highest analgesic doses, some rats were slightly ataxic and/or hyperactive. Further increase in the analgesic dosage of either drug resulted in a state of catatonia characterized by varying degrees of immobility, motor rigidity, a reduction of response to stimuli but with retention of the righting reflex. Although the rats are still analgesic during the catatonic state, this combined state does not fit our criteria for analgesia. Thus, the analgesic dose range for ketamine or morphine consisted of those doses which induce analgesia without catatonia.

Analgesia was assessed by determining the latency of response to a nociceptive stimulus (Fennessy and Lee, 1975). The distal 5 cm of the rat tail was placed in a beaker containing water at 55°C (±0.5°C) and the latency determined for the rat to flick the tail out of the water. This test was repeated three times at 15-min intervals to establish the predrug control and 6 times at 15-min intervals postdrug. To prevent tissue injury, the tail was not allowed to remain in the water for longer than 15 sec (or 10 sec for daily dose-tolerance studies). Analgesia for each animal was expressed as the TFLD between the maximal postdrug latency and the predrug average latency. The maximal TFLD occurred 30 to 45 min after drug administration (fig. 1). The criterion for analgesia was a postdrug latency which was greater than two times the predrug average latency (Janssen *et al.*, 1963). Therefore, any TFLD equal to or greater than 2.83 sec (the average predrug latency) was considered to represent significant analgesia.

The drugs and dose ranges tested were: 0.9% saline, 1 ml/kg; ketamine, 5 to 45 mg/kg; morphine, 1 to 10 mg/kg; and naloxone, 5 μg to 10 mg/kg. Naloxone was administered immediately after the first 15-min postdrug latency test to synchronize the time of peak naloxone effect with the expected peak action of the analgesic drug.

Catatonia. In this study catatonia is defined as a state in which the rat is observed to be partially or completely immobile, often with an abnormal posture but retaining the righting reflex. The neurophysiological characteristics of the catatonic state have been described previously (Winters, 1976).

Catalepsy. Further increase in dosage resulted in the loss of the righting reflex and loss of responsiveness to noxious or other stimuli. The neurophysiological characteristics of this cataleptic state also have been described (Winters, 1976). Catalepsy is defined as a state in which the rat maintains catatonia but, in addition, loses the righting reflex. The latency from time of drug administration to the onset of loss of the righting reflex was obtained for each group and the latency times averaged. Assessment of the duration of catalepsy was made by determining the DLRR. The DLRR was defined as the time interval between the loss of the righting reflex and the return of the reflex associated with ability to walk forward on all four feet. The tail of each rat was

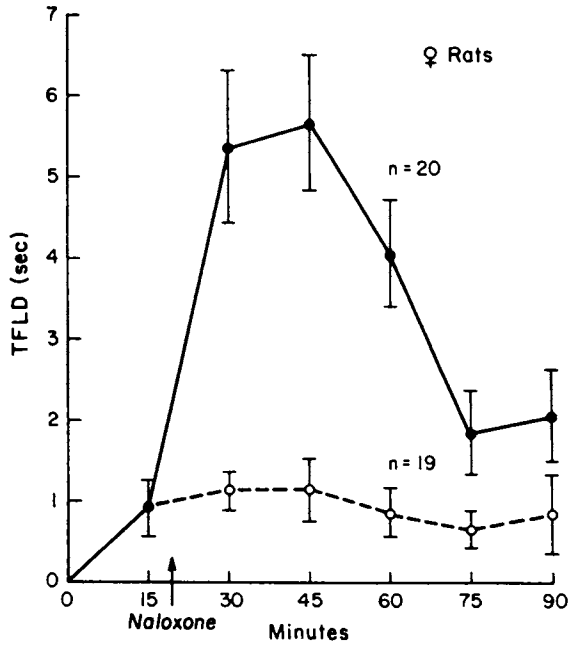

Fig. 1. The time course of action of ketamine (45 mg/kg i.p.) (●) and ketamine (45 mg/kg i.p.) + naloxone (10 mg/kg i.p.) (○) on TFLD in the female rat. Each point represents the mean ± S.E. (n = 19–20).

firmly tapped at intervals of 5 min and more frequently as recovery approached. The approach of recovery was characterized by a sequence of events: rolling from supine to prone position with limbs extended and head lying on table; flailing limb movements; circling without limbs supporting body; hind limbs flexed under body but forelimbs still extended sideways; forelimbs flexed and supporting body, head raised; two or more steps taken with body raised; the last was taken as the endpoint. The DLRR values for each treatment group were averaged. The behavior elicited during recovery was observed and recorded, *e.g.* ataxia, urination, skin color, respiratory distress and piloerection.

The dose ranges utilized for the DLRR studies were: ketamine, 50 to 80 mg/kg; morphine, 20 to 50 mg/kg; and naloxone, 5 μg to 40 mg/kg. Naloxone was administered immediately after the agonist drug.

Agonist/antagonist interactions. Percentage of inhibition of the TFLD or DLRR maximal response after naloxone was determined utilizing: ketamine, 45 and 80 mg/kg; and morphine, 10 and 50 mg/kg. The dose of naloxone that inhibited each response by 50% (ID_{50}) was determined.

Tolerance. The TFLD during daily administration of 2 mg/kg of morphine or 40 mg/kg of ketamine was determined for 3 or 5 consecutive days (respectively).

The DLRR was determined after daily administration of 45 mg/kg of morphine or 80 mg/kg of ketamine for 3 or 5 consecutive days.

Cross-tolerance. Naive rats received either 80 mg/kg of ketamine or 45 mg/kg of morphine and the DLRR measured as described. The basic protocols were as follows: 1) ketamine for 3 days with morphine on Day 4; 2) morphine for 3 days with ketamine on Day 4; 3) morphine for 3 days with ketamine on Day 5; 4) ketamine for 1 day with morphine on Day 2; 5) morphine for 1 day with ketamine on Day 2; and 6) morphine for 1 day with ketamine on Day 3.

Drug interaction. Morphine (2, 5 or 10 mg/kg) was administered to groups of rats (n = 6–10) at the same time that rats received ketamine (80 mg/kg) and the DLRR was determined as described.

Morphine plasma and brain levels. Four groups (n = 10) of rats received morphine (1, 5, 10 or 45 mg/kg) and were sacrificed 1 hr later. An additional group (n = 10) received morphine (45 mg/kg) and were sacrificed 24 hr later. A sixth group (n = 10) received morphine (45 mg/kg daily for 3 consecutive days) and were sacrificed on Day 4. Free morphine was determined in plasma and whole brain by a slight

modification of the Abuscreen radioimmunoassay method for morphine (Roche Diagnostic Systems, Nutley, NJ).

Statistical procedures. A mean and S.E. were calculated for each experimental group. Statistical comparisons between selected pairs of means were made by Student's t test; multiple comparisons to any particular group were avoided. Values of "t" with a probability of less than .05 were considered to indicate a significant difference between means.

Results

Analgesia TFLD

The mean latency to tail-flick before drug administration (predrug average latency) was determined in 294 rats to be 2.83 sec ± 0.16 (95% CL, 2.52–3.14). The value 2.8 sec was subtracted routinely from the postdrug tail-flick latencies to determine the TFLD.

Saline control. The tail-flick reaction time of 38 rats receiving 1 ml/kg of 0.9% saline was 3.1 sec ± 0.06 (95% CL, 2.98–3.22), not significantly different from the predrug average latency (P = .7).

Naloxone control. Ten rats were given 10 mg/kg of naloxone. The postdrug latency was 3.2 sec ± 0.08 (95% CL, 3.02–3.38) which was not significantly different from either predrug average latency (P = .7) or postsaline latency (P = .7).

Drug tolerance controls. After three to five daily doses of morphine or ketamine the predrug latencies did not differ significantly from the predrug average latency (2.83 sec).

Ketamine. The time course of TFLD response at control and at consecutive postdrug intervals after ketamine (45 mg/kg) can be seen in figure 1. A dose-related increase in TFLD was observed after ketamine (30, 40 and 45 mg/kg) (fig. 2B). At the 40- and 45-mg/kg doses, responses were significantly different from the saline controls (P < .02). Naloxone significantly reduced the TFLD after ketamine (45 mg/kg) (P < .001) and the ID_{50} was 6.8 mg/kg (fig. 3). At ketamine (45 mg/kg or below), none of the rats showed catatonia, although at the 45-mg/kg dose a few did exhibit some ataxia and hyperactivity (fig. 2B).

Morphine. A dose-related increase in TFLD was observed after morphine (1, 5 and 10 mg/kg) (fig. 2A). Naloxone significantly (P < .001) reduced the TFLD at each of these dose levels. The ID_{50} was 6.5×10^{-3} mg/kg when tested with morphine (10 mg/kg) (fig. 3).

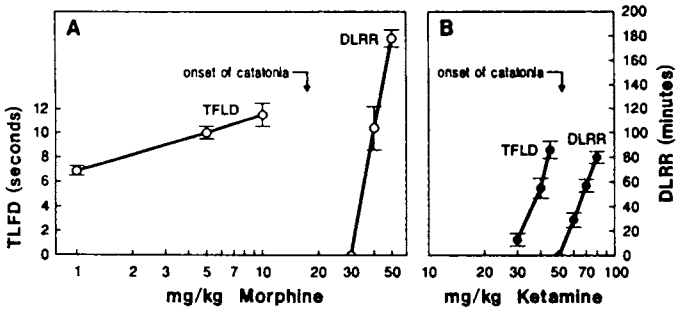

Fig. 2. Dose-response relationships of: A, morphine (○); and B, ketamine (●) on the TFLD (left scale) and on the DLRR (right scale) in the female rat. The vertical arrow at the top of each figure indicates the lowest dose level at which catatonia is seen in ketamine- or morphine-treated rats. Each point represents the mean ± S.E. (n = 5–15).

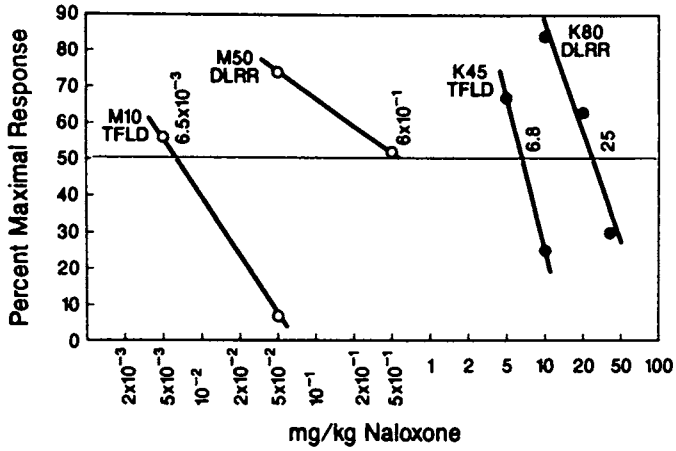

Fig. 3. Dose-dependent antagonism by naloxone of the effects of morphine (10 mg/kg i.p.) on the TFLD (M10) and (50 mg/kg i.p.) on the DLRR (M50) compared with naloxone antagonism of the effects of ketamine (45 mg/kg i.p.) on TFLD (K45) and (80 mg/kg i.p.) on DLRR (K80). The fine, horizontal line represents the 50% inhibition level, whereas the vertical numbers indicate the naloxone 50% inhibitory dose (ID_{50}), in milligrams per kilogram, for the adjacent variable. Each point represents a mean (n = 5–15).

TABLE 1

Levels of free morphine in plasma and brain after i.p. administration

Mean measured levels, ± S.E.

	Measured Levels of Free Morphine	
	Plasma	Brain
	ng/ml	*ng/g*
24 hr after 3 daily doses, 45 mg/kg	25.85 ± 4.58	16.31 ± 3.39
24 hr after 1 dose, 45 mg/kg	51.55 ± 11.81	15.95 ± 3.46
1 hr after 1 dose, 2 mg/kg*	59.01 ± 21.80	18.24 ± 10.54

* Predicted from 1 hr plasma and brain level regression lines (fig. 8).

Catatonia

Ketamine. Rats receiving doses above 45 mg/kg and less than 60 mg/kg were catatonic without loss of the righting reflex (fig. 2B).

Morphine. Rats receiving doses above 18 mg/kg and less than 40 mg/kg were catatonic without loss of the righting reflex (fig. 2A).

Catalepsy DLRR

Ketamine. A dose-related increase in DLRR was noted after ketamine (60 to 80 mg/kg) (fig. 2B). The 50-mg/kg dose induced catatonia but did not induce a loss of the righting reflex whereas the 60-mg/kg dose induced a significant DLRR in all of the rats. The DLRR of the 60-, 70- and 80-mg/kg doses were significantly different from the saline controls (P < .05). After administration of ketamine (80 mg/kg) the latency interval until loss of the righting reflex was 2.4 ± 0.23 min (table 1). Catalepsy was associated with semimalleable rigidity and during recovery the rats were ataxic and diuresed. Naloxone significantly reduced the DLRR after ketamine (60–80 mg/kg) (P < .001) and the ID_{50} against ketamine (80 mg/kg) was 25 mg/kg (fig. 3). In addition to reducing latency, naloxone also reduced the incidence of catalepsy by 30% at the ketamine 70-mg/kg dose.

Morphine. After morphine (40 or 50 mg/kg) there was a dose-related increase in the DLRR (P < .001; fig. 2A). After

morphine (45 mg/kg) the latency was 15.4 ± 0.3 min . Catalepsy was associated with a lead-pipe rigidity and no diuresis or ataxia was seen during recovery. Naloxone significantly reversed the morphine DLRR responses and the ID_{50} against morphine (50 mg/kg) was 0.6 mg/kg (fig. 3).

Tolerance Studies

Analgesia. Daily administration of either morphine or ketamine resulted in a significant daily reduction in the effect on TFLD (fig. 4, A and B). The TFLD response was reduced to less than 50% on Day 3 in both morphine ($P < .01$) and ketamine ($P < .05$) groups. Even when no ketamine was administered on Day 4, the response on Day 5 was lower than on Day 3 ($P < .001$; fig. 4A).

Catalepsy. Daily administration of either morphine (45 mg/kg) or ketamine (80 mg/kg) demonstrated a significant daily reduction in DLRR. The DLRR response dropped below 50% maximum by Day 3 in both drug groups (fig. 5, A and B).

Cross-Tolerance Studies

After 3 days of ketamine (80 mg/kg), the DLRR effect of morphine (45 mg/kg) was reduced to 57% of that expected for morphine in a naive rat ($P < .05$; fig. 5A). Surprisingly, after 3 days of morphine (45 mg/kg), administration of ketamine (80 mg/kg) induced a DLRR apparently longer than the expected Day 1 level although this was not statistically significant (fig. 5B). The augmentation of the DLRR response also was shown in these morphine-tolerant rats after morphine (45 mg/kg) on Day 10 (fig. 5C): the effect of ketamine (80 mg/kg) was augmented by 20% above control but this was not significant. The following day (Day 12) the DLRR response to ketamine (80 mg/kg) dropped to 40% of control ($P < .001$) rather than the expected drop to 60%, suggesting that some tolerance to ketamine was already present on Day 11, but was masked by the augmentation.

In rats that received morphine (45 mg/kg) for 3 consecutive days, but no drug on Day 4, ketamine (80 mg/kg) on Day 5 induced a DLRR which was not significantly less than Day 1 control (fig. 5D).

Tolerance to morphine could be demonstrated on Day 2 after a single injection of ketamine (80 mg/kg) on Day 1, as shown in figure 6A ($P < .001$). After a single dose of morphine (45 mg/kg) on Day 1, ketamine on Day 2 (fig. 6B) showed an augmented DLRR over control (fig. 6A, Day 1, $P = .05$). In another series (not shown) replicating this drug sequence, there was a 62% augmentation over the control ketamine response ($P < .001$). Tolerance to a single dose of morphine was evident when morphine was given on Day 3 after a drug-free Day 2 ($P < .001$; fig. 6C). Augmentation of the ketamine-induced DLRR on Day 4, after morphine on Day 3 (fig. 6C), was not significant. When ketamine was given on Day 3 after morphine on Day 1 (fig. 6D), a slight degree of tolerance was noted which was not significant when compared with the Day 1 ketamine control; however, the Day 3 response was significantly less ($P < .01$) than the augmented response of Day 2 (fig. 6B).

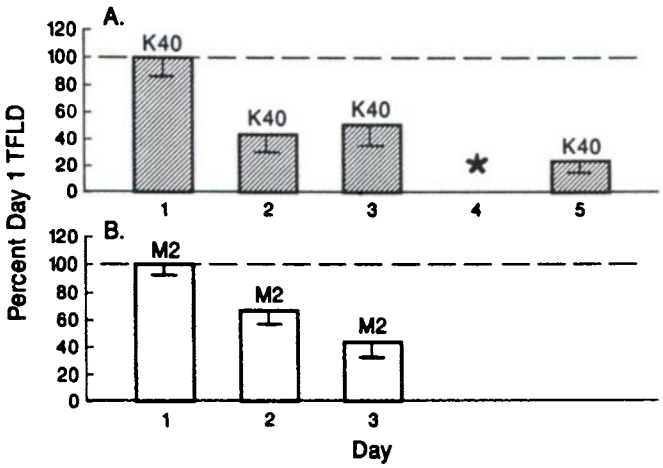

Fig. 4. Effects of once-daily injections of: A, ketamine (40 mg/kg i.p.) (K40); or B, morphine (2 mg/kg i.p.) (M2) on the TFLD, tested 30 to 45 min after drug injection (*i.e.*, at the peak of drug action). Each block represents the mean ± S.E. ($n = 10$), normalized to the Day 1 response (100%). Drug treatment and testing were omitted on Day 4 of the ketamine protocol (asterisk).

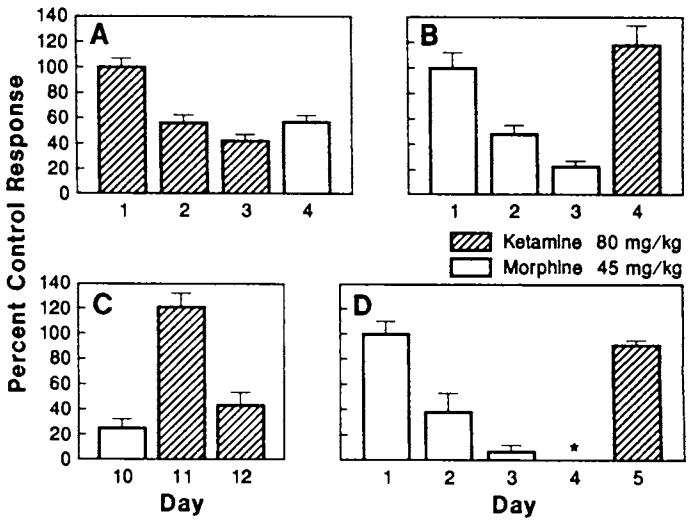

Fig. 5. A, DLRR response to morphine (45 mg/kg i.p.) in ketamine-tolerant rats (ketamine, 80 mg/kg i.p. once daily for 3 days). Ketamine responses are normalized to the Day 1 ketamine response whereas the Day 4 morphine response is normalized to the mean of a separate group of naive rats given the same dose of morphine. Mean ± S.E. ($n = 10$). B, DLRR response to ketamine (80 mg/kg i.p.) in morphine-tolerant rats (morphine, 45 mg/kg i.p. once daily for 3 days). Morphine responses are normalized to the Day 1 morphine response whereas the Day 4 ketamine response is normalized to the mean of a separate group of naive rats given the same dose of ketamine. C, after 5 days without drug, the rats from B received morphine (45 mg/kg) on Day 10 and ketamine (80 mg/kg) on Day 11 and on Day 12. Data are normalized as in B. D, after 3 days of morphine (45 mg/kg/day) drug treatment and testing were omitted on Day 4 (asterisk). On Day 5, ketamine (80 mg/kg) was given. Data are normalized as in B.

Drug Interactions

Rats receiving morphine (2, 5 or 10 mg/kg) concurrently with ketamine (80 mg/kg) demonstrated an augmented DLRR compared with ketamine control (fig. 7). The DLRR in the group receiving morphine (2 mg/kg) plus ketamine (80 mg/kg) was augmented 46% over control values ($P < .001$), similar to the DLRR augmentation noted during ketamine-morphine cross-tolerance studies (figs. 5 and 6). An augmentation of DLRR also was seen in rats made morphine-tolerant by two successive daily administrations of morphine (45 mg/kg). Forty-eight hours after the second injection of morphine, morphine (2 mg/kg) plus ketamine (80 mg/kg) produced a DLRR 21% greater than that of the ketamine control ($P < .001$).

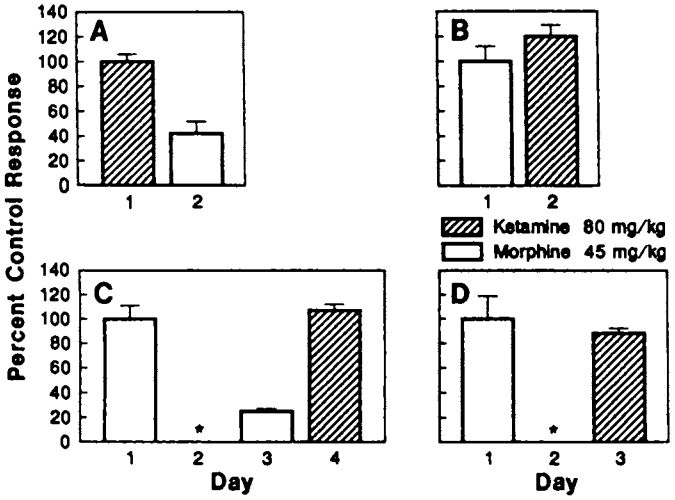

Fig. 6. A, DLRR response to morphine (45 mg/kg i.p.) 24 hr after a single dose of ketamine (80 mg/kg i.p.). Normalization as in figure 5. Mean ± S.E. (n = 10). B, DLRR response to ketamine (80 mg/kg i.p.) 24 hr after a single dose of morphine (45 mg/kg i.p.). C, after morphine (45 mg/kg i.p.) on Day 1, drug treatment and testing were omitted on Day 2 (asterisk). A second dose of morphine was given on Day 3. On Day 4, ketamine (80 mg/kg i.p.) was given. D, after morphine (45 mg/kg) on Day 1, drug treatment and testing were omitted on Day 2 (asterisk) and ketamine (80 mg/kg) was given on Day 3.

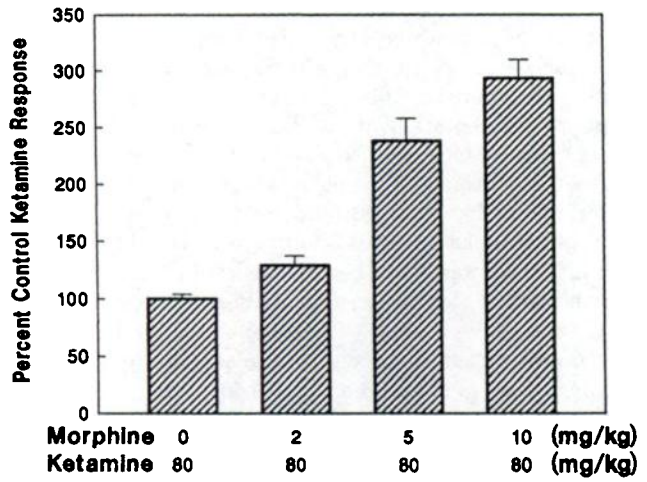

Fig. 7. DLRR dose-response relationship of morphine with a fixed dose of ketamine (80 mg/kg). Ketamine was administered simultaneously with morphine (0, 2, 5 or 10 mg/kg). Normalization to the response to ketamine alone. Mean ± S.E. (n = 10).

Morphine Levels

The plasma and brain levels of morphine 1 hr after administration increased linearly with dose (fig. 8). Plasma levels were approximately 2 times greater than brain levels at all doses. The brain concentrations of morphine 24 hr after a single dose of 45 mg/kg did not differ significantly from those 24 hr after the last of three consecutive daily doses of 45 mg/kg (table 1). The plasma level after three doses was only half of that measured after a single dose and this difference was significant (P = .05). The 24 hr postdrug values were markedly lower than the 1 hr postdrug values for both plasma and brain. However, these 24 hr postdrug values were comparable to the 1 hr values predicted from the regression lines of figure 8 for a dose of 2 mg/kg of morphine (table 1).

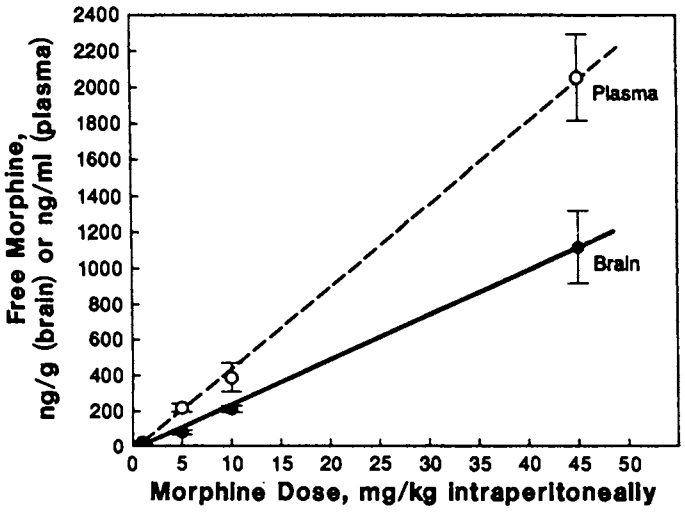

Fig. 8. Free morphine plasma and brain levels 1 hr after administration of morphine (1, 5, 10 or 45 mg/kg i.p.) to female rats. Mean ± S.E. (n = 10–20). Regression lines fitted to data points. Plasma: slope = 46.26; y intercept = −33.51; correlation coefficient = 0.999. Brain: slope = 25.50; y intercept = −32.75; correlation coefficient = 0.999.

Discussion

Ketamine and morphine both demonstrate a dose-related analgesic response as shown by the TFLD technique. Morphine is about 50 times more potent than ketamine in inducing analgesia (fig. 2). Furthermore, the analgesia induced by both drugs was reversed by the specific opioid antagonist naloxone. The ID_{50} of naloxone against morphine was at least 3 orders of magnitude lower than against ketamine (fig. 3). The naloxone reversal of the analgesic action of ketamine, a nonopioid agent, is similar to the nonopioid antagonistic effect on nitrous oxide analgesia reported by Berkowitz *et al.* (1976) and ketamine analgesia (Lakin and Winters, 1979; Smith *et al.*, 1980). Berkowitz *et al.* (1976) suggested that nitrous oxide might act by inducing the release of endogenous analgesic agents such as β-endorphins which then act at the opiate receptor to induce analgesia. This would explain both the opioid action of nitrous oxide and the lingering analgesia which they noted 30 min after nitrous oxide washout had occurred. Inasmuch as Winters *et al.* (1984) demonstrated that ketamine action was augmented rather than reduced by hypophysectomy in the chick, ketamine does not appear to act by directly releasing pituitary peptides such as β-endorphin. More likely, ketamine acts by an action on analgesia-inducing opioid receptors or by releasing β-endorphins and/or other opioid agonists from other brain regions. Although the reversal by naloxone does not of itself prove a direct opioid activity, it strongly suggests that the final action induced by ketamine involves opioid interactions.

Both ketamine and morphine induce catatonia and then catalepsy with increasing dosage. These findings are in agreement with the concept of a dose-related continuum of CNS states of excitation described previously (Winters, 1976). Doses of ketamine which produced catalepsy were only slightly in excess of those that produced analgesia without catatonia. On the other hand, there was a 3-fold difference between the highest analgesic dose of morphine which did not produce catatonia and that which produced catalepsy. Morphine (40 mg/kg) and ketamine (80 mg/kg) induced a comparable DLRR, indicating that morphine is twice as potent as ketamine. Be-

haviorally the characteristics of the catalepsy were quite different for each drug. For example, ketamine induced a semimalleable rigidity with diuresis and ataxia during recovery whereas morphine induced a lead-pipe rigidity and there was no diuresis or ataxia on recovery. This suggests that, although ketamine and morphine may overlap in inducing catalepsy, each produces discrete, adjunct effects as well.

Whereas both ketamine and morphine induce a cataleptic anesthetic state, authors refer to these states in a variety of different terms. This confuses the understanding of these induced states and related drug actions. The immobile state induced by morphine is variously termed catatonia, catalepsy, akinesia or rigidity (Havemann and Kuschinsky, 1982) and rarely is the presence or absence of the righting reflex considered. Inasmuch as catalepsy is defined classically as a state in which the subject does not respond when placed in unusual positions (Klockgether *et al.*, 1985), it is not clear how the subject can be cataleptic and still have a righting reflex.

The ID_{50} of naloxone against morphine-induced catalepsy is about 40 times less than that against ketamine, suggesting a difference in sites of action. Whereas the naloxone reversal of morphine catalepsy is well documented, the present study supports our preliminary report (Lakin and Winters, 1979) that naloxone reversed the catalepsy induced by ketamine. De Simoni *et al.* (1983) reported the naloxone reversal of ketamine analgesia but found no blockade of ketamine-induced loss of righting reflex. The doses used were not stated but must be presumed to be lower than those used in the present study. In addition, De Simoni *et al.* (1983) showed that naloxone completely prevented morphine or ketamine enhancement of dopamine metabolism in the brain and suggested that this effect was due to an interaction with the opiate receptor.

Tolerance of TFLD and DLRR to daily doses of drug were demonstrated for ketamine and morphine and the slopes of the tolerance development were similar, further underlining a possible parallelism between their mechanisms of action. Tolerance to analgesia is a well-documented phenomenon after multiple doses of opioids such as morphine (Nakamura and Winters, 1973). Tolerance to the cataleptic response of ketamine has likewise been reported (Cumming, 1976; Douglas and Dagirmanjian, 1975) as in the present study. Cumming (1976) reported that rats tolerant to ketamine have higher brain levels of ketamine than nontolerant rats upon regaining the righting reflex. This is interpreted as indicating that there is a cellular tolerance which decreases the ketamine-induced catatonia, rather than an increase in drug metabolism. These observations suggest that similar receptor mechanisms might be operational in tolerance development for each agent and for both analgesia and catalepsy.

In the present study, cross-tolerance was seen when morphine was administered on the day after ketamine. This finding further supports the premise that similar receptor mechanisms exist for morphine and ketamine. The locomotor studies of Hynes and Berkowitz (1978) also demonstrated that mice tolerant to morphine were cross-tolerant to ketamine. However, in the present study, cross-tolerance was not seen when ketamine was administered on the day after morphine. On the contrary, the ketamine-induced DLRR was augmented by the prior administration of morphine. Whether the rats were morphine-tolerant as a result of a single injection (fig. 6B), three daily injections (fig. 5B) or more (fig. 5C) was irrelevant. The duration of the cataleptic effect of ketamine was prolonged beyond that expected of a ketamine-naive rat as long as the ketamine was given no later than 1 day after the morphine. If 2 days elapsed between morphine and ketamine administrations, then no augmentation was apparent and some degree of ketamine tolerance was suggested (fig. 6D). Two separate processes appear to be at work: the development of tolerance and cross-tolerance, which may imply ketamine and morphine actions on a common mechanism, and secondly, a time-dependent potentiation of ketamine action by morphine. The interaction between morphine and ketamine is not a simple cross-tolerance, but appears to involve more complex interrelationships which require further study.

The studies of Bowyer *et al.* (1983) and Marietta *et al.* (1976) suggest that after 24 hr there would be only trace amounts of ketamine and metabolites present in rat brain. On the other hand, the present study demonstrated a significant level of free morphine in the rat brain and plasma 24 hr after a dose of 45 mg/kg. These residual levels appeared to be equivalent to the brain and plasma levels which would be expected 1 hr after morphine (2 mg/kg) (table 1). The small concentration of morphine remaining in the brain 24 hr after the 45-mg/kg cataleptic dose may augment the ketamine response. The combination of morphine (2 mg/kg) with ketamine (80 mg/kg) administered to naive rats produced an augmented DLRR as predicted. These are not strictly comparable drug treatments as the simultaneous administration of morphine with ketamine was to naive rats whereas the 24-hr residual concentration of morphine was in morphine-treated and hence presumably morphine-tolerant rats. In fact, the DLRR was augmented in morphine-tolerant rats also. These data suggest further that morphine augmentation of the DLRR response to ketamine can occur independently of tolerance to morphine. Inasmuch as the DLRR responses in these two groups were found to be similar, it must be assumed that the degree of cross-tolerance of morphine to ketamine is small. An interesting, but unexpected, finding was that the plasma level of morphine 24 hr after the last of three successive daily doses of morphine (45 mg/kg) was only one-half that seen 24 hr after a single dose. Careful review of the data suggests no explanation for this.

Clinical tests to determine whether ketamine had any influence on the withdrawal syndrome of opiate-dependent subjects were performed by Blachly *et al.* (1974). In spontaneous opiate withdrawal as well as low-dose, naloxone-induced withdrawal, ketamine produced a rapid relief of symptoms. This response is similar to that which would be expected after morphine or other opioids.

Smith *et al.* (1980) investigated the binding affinity of ketamine with rat brain membrane opiate receptors. They reported that ketamine is agonistic at the opiate receptor and ketamine inhibits binding of naloxone in the opiate binding assay. In addition, the ketamine binding was found to be stereospecific, the (+)-isomer being more potent than the (−)-isomer. This is in agreement with Ryder *et al.* (1980) who reported that the (+)-isomer was more analgesic than the (−)-isomer. Further studies by Pekoe and Smith (1982) and Finck and Ngai (1982) substantiated the binding affinity of ketamine with the opiate receptors. At present, opioid receptors have not been subclassified sufficiently to permit a presumption that analgesia and catalepsy are associated with receptors in the same or different brain loci. However, this growing collection of data indicates an overlap in the actions of morphine and ketamine. It appears likely that an interaction of ketamine with opioid receptors

mediates a major portion of the ketamine-induced analgesia and catalepsy. The induction by ketamine and morphine of analgesia, catatonia, catalepsy, tolerance, cross-tolerance, cross-potentiation and the reversal of some of these actions by naloxone all lead to the conclusion that the underlying mechanisms of action are interrelated. It is not possible to propose a simple model to explain the ketamine-morphine interactions at this time.

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