SYNTHETIC OPIOIDS COMPARED WITH MORPHINE AND KETAMINE: CATALEPSY, CROSS-TOLERANCE AND INTERACTIONS IN THE RAT

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Summary—Previously it has been shown in rats that both ketamine and morphine induced analgesia and, at larger doses, catalepsy and loss of the righting reflex, all of which were reversed by naloxone at widely different doses. Tolerance developed rapidly to either ketamine or morphine and there was cross-tolerance from ketamine to morphine. However, morphine potentiated the cataleptic effect of ketamine, whether fully-effective doses of morphine were given before ketamine or subeffective doses of both were given concurrently. The present study extends these observations to three specific mu-receptor agonists (sufentanil, fentanyl and alfentanil) and two mu- and kappa-agonist, mu-antagonist opioids (nalbuphine and butorphanol). All five of these opioids potentiated the cataleptic effect of ketamine. Each of the three specific mu agonists showed rapid development of tolerance. Fentanyl and alfentanil showed mutual cross-tolerance with ketamine, but sufentanil did not. This lack of sufentanil-ketamine cross-tolerance may reflect separation of the sites of agonist action and the sites of development of tolerance for the opioids and for ketamine. The potentiating effects of nalbuphine and butorphanol suggest that they potentiate ketamine-induced catalepsy, either by kappa-receptor interactions or by a mu agonist effect. It is suggested that the cataleptic effect of a combination of individually-subeffective doses of ketamine and morphine, rather than ketamine and one of the synthetic opioids, might be of more potential clinical usefulness.

Key words—catalepsy, tolerance, ketamine, sufentanil, fentanyl, alfentanil, nalbuphine, butorphanol.

Recently, this laboratory reported (Winters, Hance, Cadd, Quam and Benthuysen, 1988) that both morphine and ketamine induced a dose-related analgesia, while larger doses of ketamine or morphine induced catatonia, then catalepsy (catatonia plus loss of righting reflex). Morphine was 50 times more potent than ketamine in inducing analgesia and, at doses which produced a comparable duration of catalepsy, morphine was twice as potent as ketamine. Naloxone reversed both the morphine- and the ketamine-induced analgesia and catalepsy (Winters *et al.*, 1988; Hance, Winters, Quam, Benthuysen and Cadd, 1989). Of further interest were observations (Smith, Pekoe, Martin and Coalgate, 1980; Smith, Bouchal, deSanctis, Monroe, Amedro, Perrotti and Crisp, 1987; Finck and Ngai, 1982) that ketamine interacted with opioid receptor binding sites. The assumption was made (Smith, Perrotti, Mansell and Monroe, 1985) that interactions with ketamine receptors might modulate pain pathways in ways similar to those of opioids. Because of the pharmacological similarities of morphine and ketamine, the interactions between various subeffective dose combinations of these drugs was investigated (Hance *et al.*, 1989). The study showed that the combination of individually subeffective doses of morphine and ketamine induced catalepsy. In addition, this study showed a complex relationship between morphine and ketamine administered together, with respect to behavior, potentiation,

antagonism, tolerance, cross-tolerance and inhibition by naloxone. The study suggested that the induced catalepsy resulted from a cascade of events, rather than a simple, activation of a single receptor and that ketamine might share with morphine, some, but not all, of these actions.

Though structurally and physicochemically dissimilar from morphine, the synthetic 4-anilinopiperidine opioids, including fentanyl, sufentanil and alfentanil are believed to share a common site of agonist action, primarily at the mu opioid receptor. However, morphine has additional receptor site interactions (Smith *et al.*, 1987), which may not be shared by these synthetic opioids. Ketamine [2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride] is a derivative of the piperidine compound phencyclidine. The overlap between the pharmacological actions of ketamine and morphine suggested that a comparison of ketamine with the synthetic opioids might shed further light on the mechanism of action of all of these agents.

The present study was designed to compare the dose–response effects, tolerance and cross-tolerance of morphine and the fentanyl series and their interactions with small doses of ketamine. Further, this study explored interactions of ketamine with the synthetic opioids, nalbuphine and butorphanol, both of which have been reported to have mu antagonist and kappa agonist properties, to induce analgesia and to

reverse morphine-induced analgesia (Schmidt, Tam, Shotzberger, Smith, Clark and Vernier, 1985; Errick and Heel, 1983). The overall goal of the present study was to further the understanding of the complex relationships between ketamine and the opioids.

METHODS

Animals

Female Sprague–Dawley rats, weighing 200–300 g, were housed two per cage and allowed water and food *ad libitum*. Cages were 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 a.m. until 6 p.m. The rats were acclimatized at least 7 days prior to being used in these studies.

Drugs

The following drugs were used: ketamine (Keto-ject, Bristol lab, La Mirada, California); morphine sulfate (Mallinckrodt, Inc., Paris, Kentucky); fentanyl, sufentanil and alfentanil (Janssen Pharmaceutica, Beerse, Belgium); nalbuphine (DuPont Pharmaceuticals, Wilmington, Delaware); butorphanol (Bristol Laboratories, Evansville, Indiana). The doses of all drugs were calculated as the base expressed as mg/kg. Each drug was dissolved in 0.9% saline and administered by the intraperitoneal route.

Doses

Log–dose response curves for the duration of the loss of righting reflex were established for each agent alone. Tolerance and cross-tolerance were determined at catalepsy-inducing dose levels of ketamine (80 mg/kg), sufentanil (0.12 mg/kg), fentanyl (0.5–1.5 mg/kg) and alfentanil (1.0–1.6 mg/kg). These doses were large enough to induce tolerance reliably, with loss of righting reflex of sufficiently long duration to permit adequate analysis, yet small enough to avoid seizure activity or death. Subcataleptic doses of sufentanil (0.012 mg/kg), fentanyl (0.1 mg/kg) or alfentanil (0.3 mg/kg) were administered concomitantly with ketamine (15–80 mg/kg). These subeffective doses were approximately 0.1 times the effective cataleptic doses. The induced effects were compared with those of a combination of a small dose of morphine (5 or 10 mg/kg) with subcataleptic doses of ketamine (10–40 mg/kg). Nalbuphine and butorphanol, in comparably-scaled doses (10 mg/kg and 2 mg/kg, respectively), were also administered together with ketamine (15–80 mg/kg). The dose–response effects of these combinations of drugs were recorded.

Duration of the loss of righting reflex—catalepsy

All tests were performed 4–8 hr after the onset of the light period. Duration of the loss of righting reflex was determined as previously described (Winters *et al.*, 1988) and defined as the interval between loss and return of the righting reflex.

Tolerance

The duration of the loss of righting reflex, induced by catalepsy-inducing doses of sufentanil, fentanyl, alfentanil or ketamine was detemined each day for 3 consecutive days. The responses on Days 2 and 3 were compared with that on Day 1.

Cross-tolerance

Rats made tolerant to ketamine by 3 once-daily administrations of a cataleptic dose (80 mg/kg), were challenged on Day 4 by a catalepsy-inducing dose of either sufentanil, fentanyl or alfentanil. The response on Day 4 was compared with the response on Day 1 to that same opioid. Similarly, rats made tolerant to one or other of the opioids, were challenged on Day 4 by a catalepsy-inducing dose of ketamine.

Statistical analysis

Means and standard errors for the duration of loss of righting reflex were calculated for each treatment group (5–11 rats). Comparisons between selected pairs of means were made by student's *t*-test. For multiple comparisons, an analysis of variance was followed by a studentized range test (Snedecor and Cochran, 1980). Linear regression was used to determine the slopes of the dose–response curves. The slopes of the regression lines were compared by analysis of variance, as above; differences of slope with a probability of >0.05 were considered not significant and the slopes were considered not to deviate from parallelism.

RESULTS

The dose–response relationships of the 4-anilino-piperidine compounds (sufentanil, fentanyl and alfentanil) and those of ketamine and morphine are shown in Fig. 1. The data on sufentanil in Fig. 1 included both summer and winter experiments, which accounts for the apparently wide variation in the data points. The regression lines for the winter values alone or summer alone were parallel with the joint regression line, shown for sufentanil in Fig. 1 (i.e. the slopes do not differ significantly, *t*-test $P > 0.05$). There was a difference of potency so that the dose of sufentanil needed to produce a given effect was 1.7 times greater in summer than in winter. The rats receiving the larger doses of sufentanil, fentanyl or alfentanil, hypersalivated and developed seizures; still larger doses resulted in mortality. Nalbuphine and butorphanol each induced a sedated hypoactive state, when given alone, but did not induce rigidity or loss of righting reflex at any dose examined and therefore do not appear in Fig. 1. The slopes of the regression lines for sufentanil, fentanyl and alfentanil did not differ from one another but differed from those for morphine and ketamine (analysis of variance).
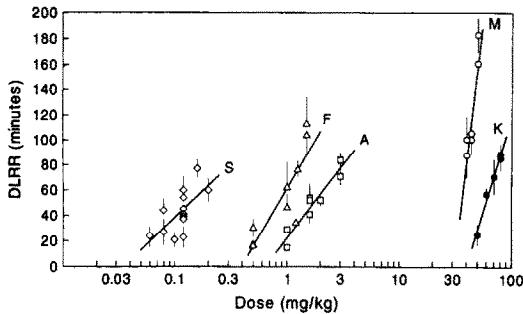
Fig. 1. Dose–response relationships, duration of loss of righting reflex (DLRR) in the female rat. Each data point represents the mean ± SE (n = 5–11). Error bars are suppressed where they are smaller than the symbol for the mean or where they would interfere with other error bars. Least-squares regression lines are shown for each drug: sufentanil (S, ◇) r = 0.665; fentanyl (F, △) r = 0.853; alfentanil (A, □) r = 0.955; morphine (M, ○) r = 0.924; ketamine (K, ●) r = 0.991. The correlation coefficient (r) is significant (P < 0.05) for all dose–response relationships.

Subcataleptic doses of ketamine, administered together with a fixed, subcataleptic dose (approximately 0.1 times the cataleptic dose) of each synthetic opioid or morphine itself, induced catalepsy (Fig. 2). Morphine and each of the synthetic opioids shifted the dose–response curve for ketamine to the left. The agonist–antagonists, nalbuphine and butorphanol, also potentiated ketamine-induced loss of righting reflex, although they induced no catalepsy when given on their own. The slopes of the linear regression lines for these ketamine-opioid combinations could not be separated from one another or from that of ketamine alone (analysis of variance, P > 0.05). The actual magnitude of the shifting to the left (potentiation) of
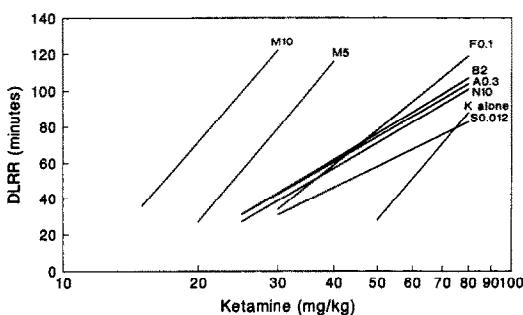

Fig. 2. Dose–response relationships, duration of loss of righting reflex (DLRR) in the female rat vs dose of ketamine in the presence of a fixed, subcataleptic dose of each of several opioids: morphine 10 mg/kg (M 10) r = 0.983; morphine 5 mg/kg (M 5) r = 0.994; fentanyl 0.1 mg/kg (F 0.1) r = 0.975; butorphanol 2 mg/kg (B 2) r = 0.958; alfentanil 0.3 mg/kg (A 0.3) r = 0.881; nalbuphine 10 mg/kg (N 10) r = 0.972; sufentanil 0.012 mg/kg (S 0.012) r = 0.945; ketamine without opioid present (K alone) r = 0.991. Only the least squares regression lines are shown, based on 4–7 doses of ketamine for each opioid. At each dose, 5–11 rats were used. The correlation coefficient (r) is significant (P < 0.05) for all dose–response relationships. The slopes of the regression lines do not differ significantly (analysis of variance, P > 0.05).
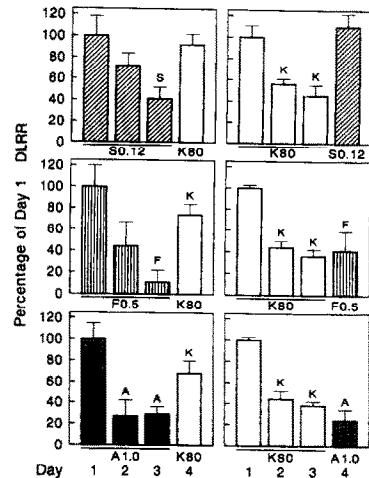

Fig. 3. Effects of once-daily administration of sufentanil 0.12 mg/kg (S 0.12), fentanyl 0.5 mg/kg (F 0.5), alfentanil 1.0 mg/kg (A 1.0) or ketamine 80 mg/kg (K 80) on the duration of loss of righting reflex (DLRR) in 6 groups of female rats. All data are normalized to the Day 1 response (100%) to the corresponding drug. Each block represents the mean ± SE (n = 9–11). Letters above a block indicate a significant difference (P < 0.05) from the response on Day 1 to the indicated drug: S, sufentanil; F, fentanyl; A, alfentanil; K, ketamine.

the dose–response curves for ketamine-induced catalepsy was dependent on the dose of each opioid used (compare morphine 5 and 10 mg/kg in Fig. 2).

Daily administration of sufentanil 0.12 mg/kg, fentanyl 0.5–1.5 mg/kg, or alfentanil 1–1.6 mg/kg, resulted in a progressive daily reduction in the duration of catalepsy, indicating the development of tolerance (Fig. 3). When a challenge dose of ketamine (80 mg/kg) was administered to these tolerant animals on Day 4, alfentanil- and fentanyl-tolerant rats were cross-tolerant to ketamine, but sufentanil-tolerant rats were not cross-tolerant. Conversely, rats receiving ketamine, 80 mg/kg daily, for three days (i.e. ketamine-tolerant) were cross-tolerant to a cataleptic dose of alfentanil or fentanyl, administered on Day 4, but were not cross-tolerant to a cataleptic dose of sufentanil.

The development of tolerance to sufentanil was repeated several times at one dose, 0.12 mg/kg. The data from all four of these replications, including the first 3 days of the cross-tolerance experiment

Table 1. Development of tolerance to sufentanil 0.12 mg/kg daily, for 3 days

| Season | n | Duration of loss of righting reflex (min), means ± SE | | |
		Day 1	Day 2	Day 3
Winter	9	60.44 ± 10.95	73.78 ± 7.04	24.78 ± 6.83*,[a]
Winter	10	53.70 ± 8.66	45.20 ± 7.38	14.70 ± 7.38*
Winter	10	44.70 ± 7.38	35.90 ± 8.16	23.50 ± 8.37*
Summer	10	39.30 ± 6.49	27.80 ± 7.82	22.40 ± 6.60*

[a]Data shown as part of Fig. 3 (as percentages of Day 1).
*Mean significantly different from mean of Day 1 (t-test, P < 0.05).

illustrated in Fig. 3, are presented in Table 1 to show their consistency. The duration of the loss of righting reflex, induced by sufentanil on Day 1, clearly showed the seasonal difference in potency described above. Irrespective of the absolute duration of the loss of righting reflex or the season of the year, a similar degree of tolerance developed over 3 days.

DISCUSSION

The present study showed that sufentanil, fentanyl and alfentanil, each induced catalepsy and tolerance and also potentiated ketamine. These synthetic opioids were reported to act with high affinity and relative selectivity at the mu opioid receptor (Leysen, Gommeren and Niemegeers, 1983; Takemori, Ikeda and Portoghese, 1986). The observed potentiation of ketamine by these opioids suggests an interaction, either at mu receptors alone, or at mu receptors in conjunction with other ketamine-activated sites (Smith et al., 1987).

Previous studies in this laboratory demonstrated that naloxone produced a shift to the right (antagonism) in the log–dose response curves for morphine, ketamine or small-dose combinations of these agents (Winters et al., 1988; Hance et al., 1989). A similar shift to the right was expected with both nalbuphine and butorphanol, but, on the contrary, these compounds potentiated ketamine in a manner similar to the potentiation shown by morphine and the 4-anilinopiperidone opioids (i.e. a shift to the left). If nalbuphine and butorphanol are indeed mu antagonists and kappa agonists (Schmidt et al., 1985), then it is possible that in augmenting the effects of ketamine, they activate kappa receptors and override the mu receptor blockade. An alternative explanation is suggested by Zimmerman, Leander, Reel and Hynes (1987). Their studies showed that nalbuphine and butorphanol are not pure mu antagonists, but rather act as partial mu agonists with high affinity for the mu receptor and reduced intrinsic activity. They demonstrated a shift to the right in the log–dose analgesia response curves for nalbuphine and butorphanol when beta-funaltrexamine, a selective and irreversible mu antagonist, was administered. The present study supports this latter hypothesis, since nalbuphine and butorphanol both potentiated ketamine-induced catalepsy.

The lack of cross-tolerance of sufentanil with ketamine was curious, since both fentanyl and alfentanil demonstrated cross-tolerance. An earlier report (Winters et al., 1988) showed that ketamine-tolerant rats were cross-tolerant to morphine, but in morphine-tolerant rats, ketamine-induced catalepsy was augmented. The presumption was that residual morphine synergized with ketamine and the resulting potentiation overrode any evidence of tolerance. It is possible that a similar explanation holds for the lack of cross-tolerance with sufentanil. Sufentanil, a very potent opioid, has been reported to show a very high

binding affinity for the mu receptor and a prolonged residual binding in brain (Leysen et al., 1983). As with morphine (Hance et al., 1989), the prolonged residual level of sufentanil in brain, at some non-tolerance-developing sites, might serve to explain the lack of cross-tolerance noted with ketamine. However, ketamine-tolerant rats were cross-tolerant to morphine, but not to sufentanil. These findings might be related to the large difference in the mu receptor binding affinity of sufentanil, as compared with ketamine. It is of interest that, despite an almost twofold winter to summer difference in the potency of sufentanil, tolerance developed at essentially the same rate and to the same degree in winter and summer months. Codd and Byrne (1981) reported a seasonal variation in the binding of various opioid agonists and antagonists in homogenates of the brain of the mouse, with the greatest binding being observed in winter and the lowest in summer. A seasonal difference in the potency of sufentanil in inducing a loss of righting reflex was noted in the present study and seasonal differences in the potency of ketamine were reported previously (Winters, Hance, Cadd and Lakin, 1986).

As postulated previously, the combination of subeffective doses of morphine and ketamine may be of clinical usefulness (Hance et al., 1989). In support of this premise is the recent report of Sperring, Sinisi, Robson, Hamilton-Dykes, Marcus, Fallon and Stanley (1988) on the clinical efficacy of ketamine, in combination with sufentanil. They showed that administration of the combination of drugs produced analgesia and loss of consciousness, with stable cardiovascular hemodynamics and without an increased incidence of postoperative respiratory depression. Similar efficacy would be predicted for combinations of ketamine with either alfentanil or fentanyl. A sufentanil–ketamine combination might be preferred in those clinical situations requiring chronic administration of drug, since this combination did not show cross-tolerance. Further studies are necessary to evaluate the potential clinical value of these ketamine–opioid combinations.

Recent studies from this laboratory have attempted to discover an underlying, unitary model to explain the interactions between ketamine and morphine, with respect to analgesia and catalepsy. The effects of a multiple-opioid-receptor agonist, morphine, and antagonist, naloxone; specific mu-receptor agonists, sufentanil, fentanyl and alfentanil; opioid mu-antagonist, kappa-agonists, nalbuphine and butorphanol; a non-opioid agonist-antagonist, ketamine, and its stereo-isomers have been studied. The results previously reported and in the present study, taken in the context of the reports of others, have not resulted in a clear picture. There are still important gaps in knowledge to be filled prior to achieving this goal.

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