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European Journal of Pharmacology 470 (2003) 149–156
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Modification of morphine analgesia and tolerance by flumazenil in male and female rats

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Received 16 January 2003; received in revised form 23 April 2003; accepted 29 April 2003

Abstract

This study assessed the effect of the central benzodiazepine receptor antagonist, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid ethyl ester (flumazenil), on morphine-induced analgesia, locomotor effects, and development of tolerance in rats. The thermally evoked pain (tail flick) response was determined after acute and chronic intraperitoneal (i.p.) administration of morphine and flumazenil, alone and in combination. In acute studies, flumazenil induced weak analgesia unrelated to dose and sex, whereas morphine-induced analgesia was dependent on both dose and sex (male>female). Flumazenil dose-dependently enhanced the analgesic effect of morphine in female but not in male rats. Isobolographic analysis suggested synergism between flumazenil and morphine in female rats, but antagonism in male rats. Flumazenil-induced locomotor changes (alone and with morphine) were related to sex but not dose. Chronic coadministration of flumazenil with morphine enhanced analgesia and attenuated tolerance development in female rats. The findings suggest a possible role for flumazenil as an adjunct with opioids in acute and chronic pain therapy.

Keywords: Flumazenil; Morphine; Tolerance; Sex; Analgesia; (Rat)

1. Introduction

Benzodiazepines have been shown to alter the analgesic action of opioids. This has been demonstrated in animal studies where the systemic administration of benzodiazepine agonists (e.g., diazepam and midazolam) attenuated morphine analgesia (Daghero et al., 1987; Luger et al., 1994; Palaoglu and Ayhan, 1986; Rosland and Hole, 1990a; Zambotti et al., 1986). Also, clinical studies suggest that benzodiazepine receptor agonists decrease morphine analgesia (Gear et al., 1997). Evidence of drugs acting at the γ-aminobutyric acid (GABA)/benzodiazepine/ionophore complex interacting with opioids is of particular interest as these drugs are frequently coadministered with opioid analgesics in the clinical setting. Much less is known about the possible interaction of benzodiazepine antagonists with opioids. Since benzodiazepine agonists seem to antagonize the analgesic effect of morphine, it seems reasonable that a benzodiazepine receptor antagonist such as flumazenil (Brodgen and Goa, 1988) might enhance morphine analgesia. Such an effect would be of significant clinical benefit, making it possible to use smaller doses of morphine to achieve similar analgesia with fewer opioid side effects (respiratory depression, sedation, constipation, and urinary retention). An initial study in humans suggests that this may occur (Weinbroum et al., 2000).

The objective of the present study was to evaluate the effect of the central benzodiazepine receptor antagonist, flumazenil, on morphine analgesia after acute and chronic administration in rats. This allowed for an evaluation of the effect of flumazenil on the acute action of morphine as well as on the development of tolerance to morphine. Both male and female rats were studied since several investigators (Cicero et al., 1996; see Kest et al., 2000 for review) have demonstrated that sex is an important factor in the analgesic response to opioid drugs. In addition, preliminary studies from our laboratory suggest sex-related differences in benzodiazepine–opioid interactions (Wala et al., 2001). Furthermore, since the responsiveness to a nociceptive stimulus may be confounded by motor dysfunction, correlation between analgesia and spontaneous motor activity was also

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doi:10.1016/S0014-2999(03)01782-5

examined. Rats were administered morphine and flumazenil, alone or in several different combinations. The responses to thermally evoked pain (tail-flick test) and changes in locomotion were assessed.

2. Materials and methods

2.1. Drugs

Morphine sulfate was purchased from Mallinckrodt (St. Louis, MO) while 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid ethyl ester (flumazenil) was a generous gift from Hoffmann-La Roche (Nutley, NJ). Drugs were dissolved in physiological saline (a few drops of 0.1 M hydrochloric acid and slight warming in a water bath were used to dissolve flumazenil). Each drug was injected intraperitoneally (i.p.) in a volume of 1 ml/kg. The dose of morphine refers to the salt form.

2.2. Care of laboratory animals

All of the experiments described herein were conducted according to a protocol approved by the University of Kentucky Animal Care and Use Committee. Rats were housed in accordance with ''The Principles of Laboratory Animal Care'' (NIH publication No. 85-23, revised 1985).

2.3. Subjects

Male and female Sprague–Dawley rats (age-matched, approximately 90 days old, weighing about 350 and 250 g, respectively, at the beginning of the experiments) served as subjects. Each rat was housed in a transparent cage with a sawdust-covered floor and had free access to standard laboratory chow and tap water in a humidity- and temperature-controlled facility with the lights on between 0600 and 1800 h. Male and female rats were kept separately housed and were tested on alternate days. Experiments were conducted during the light phase of the cycle (approximately between 0900 and 1400 h). Rats were tested each day in the same order. Body weights were recorded on the day of the experiment. The phase of the estrous cycle was not determined during the study. At the end of the experiment, the rats were killed with pentobarbital sodium (120 mg/kg, i.p.).

2.4. Tail-flick test

Nociception was assessed by the determination of the tail-flick latency using a standard tail-flick apparatus (EMDIE Instrument, Roanoke, VA). The temperature was adjusted to give control values of 2–3 s. To avoid damage to the tail, the cutoff time for heat application was set to 10 s. The rats were allowed to become familiar with the procedure prior to initiation of the study and were gently restrained during the test. The tail was blackened with ink, approximately 2–3 in. in length, 1 in. from the tail base. The thermal stimulus generated by a radiant heat source was applied to the blackened section of the tail. Lateral movement of the tail from the light source was considered a response. Each test session began with a determination of the baseline tail-flick latency taken twice, approximately 15 min apart. Baseline (preinjection) responses to thermally evoked nociception were not significantly different between male and female rats (tail-flick latency = 2.5 ± 0.60 vs. 2.5 ± 0.70 s, respectively). Testing was repeated at 5, 15, 30, 60, and 120 min after injection (acute morphine, flumazenil, morphine + flumazenil in fixed-dose ratio; chronic morphine and morphine + flumazenil) or at 15 and 30 min (graded doses of flumazenil + fixed dose of morphine). The analgesic effects were calculated as the percentage of maximum possible effect=[(postinjection tail-flick latency − preinjection tail-flick latency)/(cutoff time − preinjection tail-flick latency) × 100]. For construction of the dose–response curves, the analgesic effect was expressed as the mean percent maximal possible effect at the time of peak effect ± S.E.M. for *n* rats/sex/treatment. The analgesic effect of graded doses of flumazenil, in combination with a fixed dose of morphine, was expressed as mean value of the percent maximal possible effect (15- and 30-min average) ± S.E.M. for *n* rats/sex.

2.5. Total locomotion

Spontaneous motor activity (total locomotion) was assessed using the Opto-Varimex infrared photocell-based activity monitor (Columbus Instruments, Columbus, OH). The beam interruptions were monitored along a single axis and spontaneous motor activity (ambulatory count and stereotypic activity) was scored during two 5-min test sessions: prior to (baseline) and at 15 min after administration of drugs. In each rat, locomotion was measured immediately after the tail-flick test. Baseline (preinjection) spontaneous motor activity was less in males than females [locomotion = 210 (140–260) vs. 248 (193–296), $P < 0.01$, Mann–Whitney rank sum test]. Scores for locomotion were normalized for baseline (postinjection locomotion − preinpreinjection locomotion). Data are presented as mean locomotion values ± S.E.M. of *n* rats/sex/treatment.

2.6. Procedures

Four groups of rats for each sex were acutely treated as follows: (1) flumazenil alone; (2) morphine alone; (3) a constant dose of morphine in combination with flumazenil in 10-fold dose increments; and (4) mixtures of morphine and flumazenil in 100:1 fixed-dose ratio combinations. Drugs were injected (i.p.) at 96-h intervals using a Latin square design to balance the order of doses within a group. Two other groups of rats for each sex (chronic study) were repeatedly administered morphine followed by either flumazenil or saline (every 24 h for 10 days).

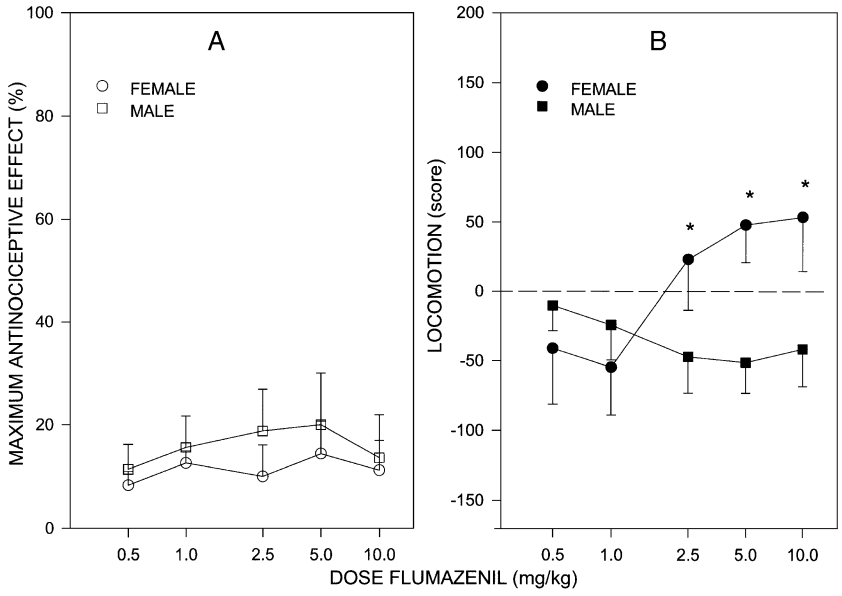

Fig. 1. Dose–response curves for analgesia (panel A) and changes in total locomotion (panel B) produced by flumazenil administered by the i.p. route in male and female rats. Analgesia is presented as the percent maximal possible effect at the peak time in response to thermally evoked nociception. Locomotion is presented as 5-min scores (normalized for baseline). Data are the mean maximum antinociceptive effect (%) or mean locomotion ± S.E.M. of 10 rats/sex as a function of the log of the flumazenil dose. *Significantly different from identically treated male rats ($P<0.05$, post-hoc Student–Newman–Keuls test).

2.7. Data analysis

The effective doses that produce 50% maximal antinociceptive effects for morphine alone ($ED_{50(morphine)}$) and for the mixture of morphine with 100-fold lower dose of flumazenil ($ED_{50(mix)}$) were calculated from the regression line (percent maximal possible effect vs. log dose), where dose represents either morphine alone or total dose of morphine plus flumazenil (Tallarida et al., 1997). The interaction between morphine and flumazenil was determined as previously described for the case when one of the two compounds (flumazenil in this case) does not have any significant efficacy alone (Maves et al., 1994). The theoretical additive value of ED_{50} and its S.E.M. was calculated as $ED_{50(add)} = ED_{50(morphine)}/P_1$ and S.E.M. $(ED_{50(add)}) =$ S.E.M. $ED_{50(morphine)}/P_1$, where P_1 was equal to the proportion of morphine in a morphine–flumazenil

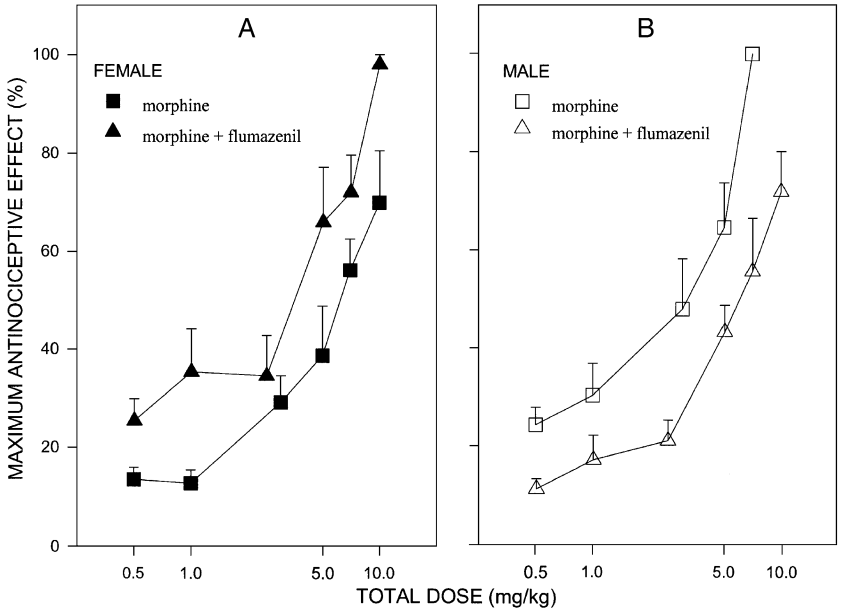

Fig. 2. Dose–response curves for analgesia induced by morphine alone and mixtures of morphine and flumazenil administered i.p. to rats in a fixed-dose ratio equal to 100:1 for morphine and flumazenil, respectively. Analgesia is presented as the percent maximal possible effect at the peak time in response to thermally evoked nociception. Closed symbols correspond to females (panel A) and open symbols to males (panel B). Data are presented as the mean maximum antinociceptive effect (%) ± S.E.M. of 10 rats/sex/treatment vs. the log dose of morphine alone or log total dose of morphine + flumazenil.

zenil mixture. Synergism was revealed when the experimental $ED_{50(mix)}$ of the mixture was smaller than the theoretical $ED_{50(add)}$ of a purely additive mixture having the same proportion. An $ED_{50(mix)}$ (experimental) greater than $ED_{50(add)}$ (theoretical) indicated an antagonistic interaction (Porreca et al., 1990).

2.8. Statistics

The valid use of parametric statistics was verified by normal distribution and equal variance (Kolmogorov–Smirnov normality test and Levine median test, $P < 0.05$). Dose–response curves were determined by linear regression. Between-doses or between-days differences were assessed with one-way repeated-measures analysis of variance (ANOVA) with post-hoc Student–Newman–Keuls test. One-way repeated-measures ANOVA on ranks and post-hoc Dunnett's method were used to analyze data that failed normality and/or equal variance tests. Between-sex differences were compared by two-way repeated-measures ANOVA (sex and dose taken as factors) with post-hoc Student–Newman–Keuls test, Mann–Whitney rank sum test, and *t* test. Level of significance was $P < 0.05$.

3. Results

3.1. The analgesic and motor effects of flumazenil

In the range of doses tested herein (0.5–10 mg/kg, i.p.), flumazenil produced a weak analgesia (Fig. 1A). Regardless of sex, the analgesic effect of flumazenil was not significantly related to dose and, thus, the ED_{50} could not be determined for flumazenil. Some motor depression was observed at lower doses of flumazenil, while enhancement was typically seen at higher doses in female rats. In contrast, there was a tendency

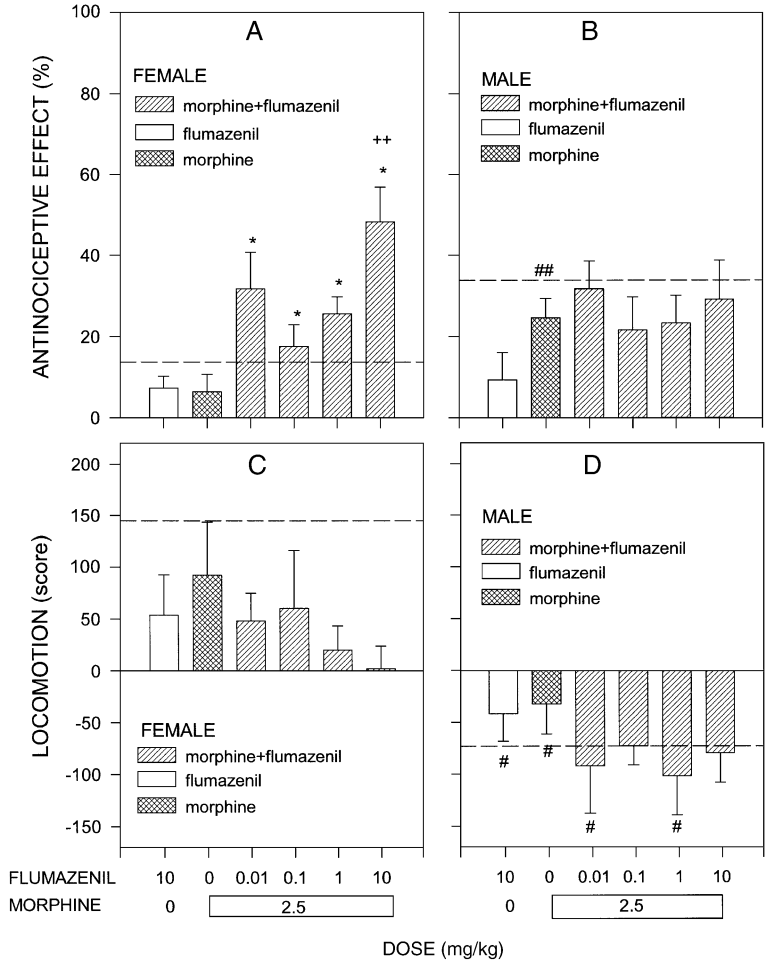

Fig. 3. The analgesic effect (panels A and B) and changes in total locomotion (panels C and D) produced by flumazenil (10 mg/kg) alone, morphine (2.5 mg/kg) alone, and graded doses of flumazenil (0.01–10 mg/kg) in combination with a fixed dose of morphine (2.5 mg/kg) administered i.p. in male and female rats. Analgesia is presented as the percentage of maximal possible effect (average at 15 and 30 min after injection). Locomotion is presented as 5-min scores (normalized for baseline). Data are the mean ± S.E.M. of 10 rats/sex. The dashed lines indicate the anticipated values for additivity. *Different from morphine alone ($P < 0.05$, post-hoc Dunnett's method). $^{++}$Different from 0.1 mg/kg flumazenil + morphine ($P < 0.05$, post-hoc Student–Neuman–Keuls test). $^{\#\#}$Antinociceptive effect different from identically treated female rats ($P < 0.01$, Mann–Whitney rank sum test). $^{\#}$Locomotion different from identically treated female rats ($P < 0.05$, *t* test).

toward a decrease of locomotion with increasing dose of flumazenil in male rats (Fig. 1B). Overall, the effect of flumazenil on locomotion was related to sex [$F(1,99) = 11.4$, $P < 0.005$, two-way repeated-measures ANOVA], but not to dose.

3.2. The analgesic and motor effects of morphine

The analgesic effect of morphine (0.25–10 mg/kg, i.p.) was significantly related to both dose and sex [$F(4,99) = 30.9$ and $F(1,99) = 14.3$, respectively, $P < 0.005$, two-way repeated-measures ANOVA] (Fig. 2A and B). The potency of morphine was greater in male than in female rats ($ED_{50} = 1.92 \pm 0.38$ vs. 6.17 ± 1.27 mg/kg, respectively, $P < 0.005$, t test). The motor effect of morphine was related neither to dose nor sex (data not shown).

3.3. Flumazenil–morphine interactions on analgesia and locomotion (acute administration)

The analgesic effect of a constant dose of morphine (2.5 mg/kg) was progressively enhanced by coadministration of increasing doses of flumazenil (0.01–10 mg/kg) in female rats (Fig. 3A). The increase in percent maximal possible effect was of statistical significance for each tested dose of flumazenil [$\chi^2 = 15.0$ ($df = 4$), $P = 0.005$, one-way repeated-measures ANOVA on ranks; post-hoc Dunnett's method, $P < 0.05$]. In contrast, flumazenil did not significantly affect morphine analgesia in identically treated male rats (Fig. 3B). Consequently, sex differences in morphine analgesia (male>female) were lost in the presence of flumazenil. Further, there was a trend for a decrease in motor activity (towards baseline) with increasing dose of flumazenil with the mor-phine–flumazenil mixture in female rats (Fig. 3C), while identical treatment attenuated locomotion in a non-dose-related manner in male rats (Fig. 3D). Overall, the addition of increasing doses of flumazenil to a fixed dose of morphine caused changes in locomotion that were related to sex [$F(1,99) = 10.0$, $P < 0.005$, two-way repeated-measures ANOVA], but not to dose of flumazenil.

The percent of maximal possible antinociceptive effect at the peak time significantly increased with increasing dose of morphine plus flumazenil in 100:1 fixed-dose ratio [$F(5,49) = 10.2$ and 6.4 in male and female rats, respectively, $P < 0.0005$, one-way repeated-measures ANOVA] (refer to Fig. 2A and B). As can be seen, flumazenil shifted the dose–response curves of morphine to the left in female rats and to the right in male rats. The experimentally determined $ED_{50(mix)} = 2.41 \pm 0.40$ mg/kg after coadministration of morphine and flumazenil was significantly lesser than the calculated $ED_{50(add)} = 6.23 \pm 1.28$ mg/kg in female rats ($P < 0.025$, t test). In contrast, the $ED_{50(mix)} = 4.95 \pm 1.15$ mg/kg was significantly greater than the theoretical additive $ED_{50(add)} = 1.94 \pm 0.38$ mg/kg calculated for morphine plus flumazenil in male rats ($P < 0.025$, t test).

3.4. Flumazenil–morphine interactions on analgesia and locomotion (chronic administration)

The present data demonstrate that regardless of sex, tolerance developed to the analgesic effect of chronically administered morphine (compare day 1 to day 10). Flumazenil attenuated tolerance to the analgesic effect of morphine in female rats (Fig. 4A). In contrast, flumazenil did not

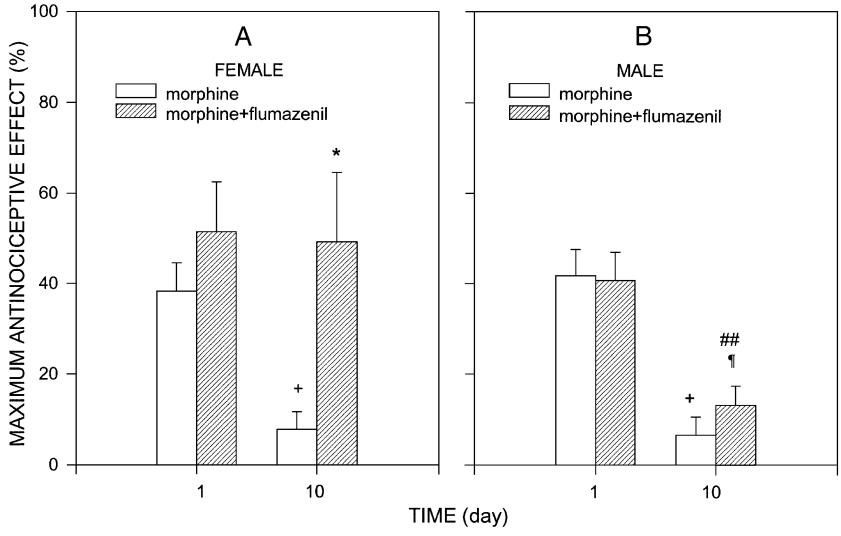

Fig. 4. Analgesia produced by repeated administration of morphine (5 mg/kg/day, i.p.) alone and in combination with flumazenil (1 mg/kg/day, i.p.) in female (panel A) and male rats (panel B). Open bars correspond to morphine alone and closed bars correspond to morphine + flumazenil. Analgesia is presented as the percent maximal possible effect at the peak time in response to thermally evoked nociception ± S.E.M. of 10 male and 8 female rats per treatment on days 1 and 10 of chronic treatment. *Morphine + flumazenil, different from morphine alone (day 10). [†]Morphine alone, day 10 different from day 1. [¶]Morphine + flumazenil, day 10 different from day 1 ($P < 0.05$, post-hoc Dunnett's method). [##]Different from morphine + flumazenil-treated female rats, day 10 ($P < 0.01$, Mann–Whitney rank sum test).

significantly affect tolerance development in male rats (Fig. 4B). Two-way repeated-measures ANOVA indicated that the analgesic effect significantly changed across time of the chronic administration of morphine or morphine + flumazenil [$F(7,143) = 7.0$, $P < 0.0001$ and $F(7,143) = 3.4$, $P < 0.005$, respectively] and was related to sex (male < female) for morphine + flumazenil [$F(1,143) = 26.7$, $P < 0.0001$], but not for morphine alone. Further, differences in the analgesic effects produced by morphine vs. morphine + flumazenil were of statistical significance in female rats only [$F(1,143) = 20.7$, $P < 0.0001$]. No tolerance to the locomotor effects of morphine was observed when morphine was administered alone or with flumazenil.

4. Discussion

The main findings in this study are as follows. First, we have demonstrated that flumazenil, by itself, produced a weak analgesia that was neither related to dose nor sex. Second, flumazenil affected the analgesic effect of morphine in a sex-related manner. Thus, the presence of flumazenil abolished the diminished response to acute morphine in female rats compared to male rats. Third, flumazenil inhibited the development of tolerance to morphine analgesia in female but not in male rats.

The first question we addressed was whether the analgesic properties of flumazenil differed between sexes. We demonstrated that flumazenil produced similar levels of weak analgesia in male and female rats. It has been previously reported that lower doses of flumazenil had modest analgesic effects while higher doses of flumazenil (up to 60 mg/kg) produced significant analgesia in male rats and mice (Cappell et al., 1989; Davidovich et al., 1988; Luger et al., 1994; Morgan et al., 1987; Walsh et al., 1986). It appears unlikely that the weak analgesia observed herein was an artifact due to a depressant effect of flumazenil on motor activity. Flumazenil-induced analgesia was accompanied by changes in motor activity that were opposite in male and female rats (a decrease and enhancement of motor activity with increasing dose of flumazenil in male and female rats, respectively). It is likely, however, that the analgesia seen at higher doses of flumazenil might be confounded by marked motor dysfunction. We have found that motor activity was markedly below baseline for a range of flumazenil doses (i.v.) equal to 10–40 mg/kg in male rats and for the 40 mg/kg dose only in female rats (unpublished data from our laboratory).

Next, we have confirmed that the analgesic effect of morphine is dose-related and that the effect is greater in male than in female rats (Cicero et al., 1996; see Kest et al., 2000 for review). Despite extensive studies on the role of hormonal status, pharmacokinetics, differences in distribution of opioid receptors, and/or neuronal circuitry, the reason for sex differences in morphine analgesia is not completely understood. It appears that intrinsic differences in CNS sensitivity may be important (Cicero et al., 1996). In addition, recent findings demonstrated that whereas sex steroids are involved in morphine-induced analgesia, it is due to their organizational effects as the brain develops rather than to their acute activational effects in adulthood (Cicero et al., 2002). The present study additionally showed that regardless of sex and initial sensitivity to morphine analgesia, a similar degree of tolerance was present after chronic administration at the 10-day time period. No apparent tolerance to the locomotor effects was seen after chronic administration of morphine alone or in combination with flumazenil.

Finally, we examined the flumazenil–morphine interaction on analgesia after acute and chronic administration in both sexes. Similar to earlier studies in male mice and rats (Brady et al., 1984; Kubota et al., 1985; Rosland and Hole, 1990a; Zambotti et al., 1986), we found no enhancing effect of flumazenil on morphine analgesia in male rats. In contrast, flumazenil potentiated morphine analgesia in female rats. Flumazenil-induced enhancement of morphine analgesia was accompanied by a decrease in motor activity in female rats; however, scores for locomotion were higher or equal to baseline. On the other hand, unchanged analgesia was accompanied by reduced locomotion after coadministration of flumazenil and morphine in male rats. Thus, it appears unlikely that the response latencies on the tail-flick test were confounded by a motor dysfunction after administration of flumazenil and morphine.

In order to more fully understand the nature of the acute flumazenil–morphine interaction, we employed isobolographic analysis (Tallarida et al., 1997). This approach confirmed and extended our previous observations. The experimentally determined ED_{50} for morphine–flumazenil mixtures (in fixed ratios) was almost threefold lower than the corresponding theoretical value based on simple additivity in female rats. This confirmed that modulation of morphine analgesia by flumazenil was not additive but synergistic and that an analgesic dose of morphine could be reduced by a dose of flumazenil that was devoid of a significant antinociceptive effect by itself in female rats. In contrast, the greater experimental ED_{50} than the corresponding theoretical ED_{50} suggested an antagonistic interaction in male rats.

The effect of flumazenil on the development of tolerance seen with chronic morphine administration was also examined in the present study. Flumazenil attenuated the development of tolerance to morphine in female rats while having little or no effect in male rats. This is a novel finding regarding flumazenil. Recent evidence concerning morphine tolerance indicates that it may involve excitatory amino acid receptor [*N*-methyl-D-aspartate (NMDA)]-mediated cellular and intracellular mechanisms (Mao et al., 1995). An action of flumazenil on NMDA receptors has been suggested (Sharma and Kulkarni, 1993; Przegalinski et al., 2000). The enhancing effect of several NMDA receptor antagonists on acute morphine analgesia has been shown to be greater in female than in male rats (Wala et al., 2003).

The present studies are not sufficient to determine the mechanisms underlying the interaction between morphine

and flumazenil. There is a line of evidence supporting the involvement of both the opioidergic and GABAergic systems in the mediation of morphine antinociception (Cohen et al., 1992; Cox and Collins, 2001; Depaulis et al., 1987; Drake and Milner, 1999; Drower and Hammond, 1988; Lopez et al., 1990; Moreau and Fields, 1986; Sivam and Ho, 1985; Stanford and Cooper, 1999). The analgesic interactions between naloxone and flumazenil (Cappell et al., 1989; Davidovich et al., 1988) as well as between flumazenil and benzodiazepines (Edwards et al., 1990; Luger et al., 1994; Morgan et al., 1987; Yanez et al., 1990) may suggest a mechanism(s) of action involving the endogenous opioid and GABAergic systems, respectively. It has been repeatedly shown that benzodiazepines attenuate morphine analgesia and that flumazenil reverses this effect (Daghero et al., 1987; Luger et al., 1994; Palaoglu and Ayhan, 1986; Rosland and Hole, 1990a,b). These observations point out the involvement of the benzodiazepine recognition site on the $GABA_A$/benzodiazepine/ionophore complex in opioid analgesia. In contrast to the antagonistic interaction between acute benzodiazepine and morphine, repeated benzodiazepine treatment prolonged morphine analgesia in rats (Tejwani et al., 1993; Wala et al., 2001). Thus, it appears that activation (by acute benzodiazepines) and functional impairment (by chronic occupation of the benzodiazepine receptors) of the GABAergic system have opposite effects on morphine analgesia. In view of the above data, it is appealing to speculate that flumazenil, acting as a benzodiazepine antagonist, modulates the effect of morphine via the $GABA_A$/benzodiazepine/Cl^- receptor complex by blocking the benzodiazepine recognition site. The present data suggest that this could be the case in female rats (enhancement of analgesia) but not in male rats, where flumazenil seems to have a benzodiazepine-like effect on morphine (attenuation of analgesia). As revealed by several behavioral tests, in addition to the antagonistic effect, flumazenil showed weak partial agonist and inverse agonist activities (see Brodgen and Goa, 1988 for review). Neuroactive steroids are known to modulate $GABA_A$ receptors (see Olsen and Sapp, 1995; Upton and Blackburn, 1997 for review). It is also likely that neurosteroid levels change flumazenil function. Interestingly, several studies in humans suggest that during premenstrual stage, flumazenil acts as an inverse agonist (Le Melledo et al., 2000; Strohle et al., 1999). Taken together, as the pharmacological properties of GABA receptors are defined by composition of subunits, treatment with morphine and flumazenil can result in sex-related compensatory adaptation in receptor function. Thus, the heterogeneity of the $GABA_A$/benzodiazepine/Cl^- receptor complex; the effect of the hormonal milieu on the rate of GABA turnover; differences in population and distribution of opioid receptors, endogenous benzodiazepines, opioid peptides, and second messengers; as well as differences in several other anatomical and physiological factors can contribute to the sexual dimorphism of the flumazenil–morphine interaction. Finally, although sex-linked pharmacokinetics cannot be ruled out, this mechanism is not likely due to the facts that flumazenil alone had a similar analgesic profile in both sexes (present data) and that despite sex-related morphine antinociception, the brain and plasma levels of morphine were similar in male and female rats (Cicero et al., 1997). Flumazenil has a very short half-life time ($\approx$ 8 min) in male rats (Mandema et al., 1991) whereas we are not aware of similar data in female rats.

The present findings regarding flumazenil–morphine interactions are of clinical interest since opioids remain the mainstay for the treatment of moderate to severe pain. However, opioid therapy is complicated by significant side effects (sedation, respiratory depression, constipation, pruritus, and tolerance to analgesia with chronic administration) (Gutstein and Akil, 2001). The combination of flumazenil with morphine might be expected to decrease opioid side effects and minimize tolerance development. An initial clinical study supports the finding of fewer opioid side effects when flumazenil is used with morphine in the acute postoperative pain setting (Weinbroum et al., 2000). Studies in chronic pain patients, where the effect of flumazenil on morphine tolerance can be better studied, remain to be done. In addition, since several human studies showed that gender might play a role in opioid efficacy (see Miaskowski and Levine, 1999 for review), it will be interesting to test whether coadministration of flumazenil, as an adjunct to morphine, is more effective in one gender vs. the other.

Acknowledgements

The authors thank Hoffmann-La Roche for the generous gift of flumazenil. The Department of Anesthesiology, College of Medicine, University of Kentucky, Lexington, KY, provided financial support for this research.

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