Animal Study

Dezocine Antagonizes Morphine Analgesia upon Simultaneous Administration in Rodent Models of Acute Nociception

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Disclaimer: This work was supported by grants from the National Natural Science Foundation of China (30972846 and 81471127) (PI: Y-X.Y.) and from the Talent Project of Zhejiang Provincial Department of Health (2012RCB018 and 2013RCA016) (PI: Y-X.Y.)
Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 06-10-2015
Revised manuscript received: 08-28-2016
Accepted for publication: 09-16-2016

Free full manuscript: www.painphysicianjournal.com

Background: Dezocine is a powerful analgesic that can be less addictive than morphine, yet how the two drugs interact in vivo is poorly understood. Here we administered dezocine alone or in combination with morphine to different acute nociception paradigms to explore the interactions of the 2 drugs upon co-administration.

Objective: To evaluate how dezocine interacts with morphine in different acute nociception paradigms.

Study Design: Laboratory animal study.

Setting: Zhejiang University School of Medicine, Hangzhou, China.

Methods: Healthy mice were treated with saline, dezocine (0.625 – 2.5 μg), or a combination of dezocine with morphine (2.5 μg). Tail withdrawal latency (TWL) was analyzed prior to and 30 minutes after drug administration. Rats were treated with saline, morphine (3 mg/kg), dezocine (3 mg/kg), or a combination of both drugs. The animals were then left uninjured, subjected to plantar incision, or underwent formaldehyde-induced acute inflammation. Nociception was then analyzed in terms of mechanical threshold (MT) to von Frey stimulation and paw withdrawal latency (PWL) to thermal stimulation. Formaldehyde-induced pain score was calculated based on the duration of biting and elevating of the animal's legs. Phosphorylation of extracellular signal-regulated kinase (pERK) was also measured after plantar incision as a molecular index of nociception.

Results: Dezocine enhanced TWL but inhibited morphine analgesia in a dose-dependent fashion in mice. Usage of morphine or dezocine alone in uninjured rats increased MT, but co-administering both drugs did not further increase MT. Usage of one drug alone, and both drugs together increased MT and PWL relative to saline at 30 minutes after incision. Usage of one drug alone, but not both drugs together, increased MT and PWL at 120 minutes after incision. Dezocine reduced formaldehyde-induced nociception but co-administering both drugs did not further reduce pain behavior.

Limitations: The results were obtained from animal study; clinical investigations will be needed to clarify their interaction.

Conclusion: Dezocine antagonizes morphine analgesia on acute nociception upon simultaneous administration.

Key words: Dezocine, morphine, acute nociception, analgesia

Pain Physician 2017; 20:E401-E409

Opioid ligands are widely used clinically for treating pain, though their physiological effects can be complex and undesirable. For example, selective μ-opioid agonists are effective antinociceptive agents, but analgesic doses can induce respiratory depression and physiological dependence (1,2). Each class of opioid receptors represents an important and unique drug target: δ-opioid receptors are responsible for analgesia, functions of the autonomic nervous and neuroendocrine systems, and mood-driven behaviors; κ-opioid receptors mediate spinal antinociception; and μ- and κ-opioid receptors, but not δ-opioid receptors, are involved with ventilatory depression (3,4). The problem with most opioid ligands, including so-called selective ones, is that they usually activate multiple receptor types to a greater or lesser degree. Thus, the in *vivo* effect of opioid drugs reflects their cumulative effect on all receptor types with which they interact.

Dezocine, a synthetic bridged aminotetralin, acts as a partial opioid μ-receptor agonist and a κ-receptor antagonist (5,6). It has been widely used perioperatively in China, Japan, and other Asian countries. Similar drugs are widely used in the US (7,8). Dezocine provides potent analgesia and is associated with minimal side effects and low risk of dependence (9,10). It is reported that 10 mg dezocine provides the similar analgesic effect as to either 50 mg meperidine or 10 mg morphine (11,12). However, interaction of the 2 drugs in *vivo* is poorly understood and appears to be complex. Animal and clinical studies indicate that using dezocine and morphine in combination produces different effects depending on administering sequence. For example, Morgan et al (13) reported that dezocine enhances the analgesic effects of morphine in rats when given after morphine, while Gal and DiFazio (14) reported that dezocine antagonizes morphine analgesia when given prior to morphine in humans. Furthermore, Strain et al (15) reported that in opioid-dependent humans, the antagonistic effect of dezocine was only slightly weaker than that of the pure antagonist naloxone. Since using multiple opioid analgesics in combination remains a common practice for dealing with perioperative pain, studies are urgently needed to clarify the effects of co-administering dezocine with a pure opioid receptor agonist like morphine in acute pain settings.

In the present study, we relied on mouse and rat models of acute nociception to unravel the complex interactions between dezocine and morphine when co-administered in *vivo*. We hypothesized that dezocine, a weak μ-receptor agonist, might antagonize the antinociceptive activity of morphine, such that combining the 2 drugs would increase opioid consumption without providing additional clinical benefit. In addition to measuring conventional behavioral indicators of pain response, including pain scores, tail withdrawal latency (TWL), mechanical threshold (MT), and paw withdrawal latency (PWL), we also examined levels of spinal phosphorylated extracellular signal-regulated kinase (pERK) in response to plantar incision. Levels of pERK are used as an indicator of neuronal activation in nociceptive pathways in the spinal dorsal horn (16,17).

Methods

Animals

Male adult C57 mice weighing approximately 25 g and Wistar rats weighing approximately 200 g were obtained from the Animal Center of the Chinese Academy of Sciences (Shanghai, P. R. China) and housed in groups of 10 mice per cage or 2 rats per cage. Water and food were supplied *ad libitum*, and a 12-hour light/12-hour dark cycle was used with lights on at 08:00 a.m. The study protocol was reviewed and approved by the Animal Care and Use Committee of Zhejiang University, and was consistent with international ethical guidelines for experimental pain studies in animals (18). Animals were acclimated in the housing facility for 3 days before experiments, and were allocated randomly into different treatment groups. Experiments were carried out with anesthetic techniques, as long as animals can be administered with anesthetic. All animals were euthanatized under an overdose of anesthetic in accordance with the ethical guidelines. All efforts were made to minimize the number of animals and their suffering.

Drug Treatment and Tail Flick Test in Mice

Mice were treated intrathecally with saline, dezocine alone (0.625 – 2.5 μg per animal; Yangtze River Pharmaceutical Group Co., Ltd., Taizhou, P. R. China), or a combination of morphine (2.5 μg; Northeast Pharmaceutical Group Shenyang No. 1 Pharmaceutical Co., Ltd., Shenyang, P. R. China) with dezocine (0.625 – 2.5 μg) per animal. TWL was measured before treatment (baseline) by immersing the tail in 48°C water as described (19). Then drugs (in a total volume of 5 μl) were intrathecally administered by direct lumbar puncture, as described by Mestre et al (20). A brief lateral flick of the tail worked as the sign of a successful puncture. The TWL was measured again 30 minutes later.

Drug Treatment and Physiological Nociception in Rats

Rats were injected intraperitoneally with one of the following: saline, morphine (3 mg/kg), dezocine (3 mg/kg), or both morphine (3 mg/kg) and dezocine (3 mg/kg). In all cases, a total volume of 240 µl was injected. Just before injection (baseline) and one hour after, the mechanical threshold (MT) to an electronic von Frey anesthesiometer stimulation was measured as described below.

Mechanical Threshold to von Frey Filament Stimulation

MT in response to mechanical stimulation was performed as described by Deseem et al (21) In brief, animals were placed in cages with a floor with wire mesh and allowed to explore and groom until they had acclimated. An electronic von Frey anesthesiometer (Model 2390, IITC/Life Science, Victory Blvd Woodland Hills, CA) with a flexible probe was applied to the hind paw plantar. The MT was automatically recorded when the rat hind paw either abruptly withdrew or made a flinching movement. For each animal, MT values were measured in triplicate and averaged.

Drug Treatment and Models of Acute Incision Pain

Thirty minutes after drug treatment, acute incision pain was induced as described by Brennan et al (22). Briefly, animals were anesthetized by inhalation of 3% isoflurane (Abbott Laboratories, Shanghai, P. R. China). The right hind paw was cleaned with povidone-iodine, and a no. 11 blade (Doublesword Co., Ltd, Lishui, P. R. China) was used to make a 1-cm longitudinal incision through the skin and muscle of the plantar aspect of the right paw. After applying gentle, homogeneous pressure at the incision site for a short period (usually less than a minute), the incision was closed using an HS-26 needle and 2 mattress silk sutures sized 5/0. Penicillin (80,000 units; North China Pharmaceutical Group, Shijiazhuang, P. R. China) was subcutaneously administered to prevent infection.

Just before drug treatment and 30 and 120 minutes after incision, MT to von Frey filament stimulation was measured, as was PWL in response to thermal stimulation (see below).

Paw Withdrawal Latency in Response to Thermal Stimulation

PWL to noxious heat stimuli was measured using an apparatus for measuring PWL (Model 336, IITC/Life Science) as described by Hargreaves et al (23). Briefly, the rat was placed in a Plexiglas chamber on a glass plate above a light box. A radiant heat stimulus was applied by directing a beam of light through a hole in the light box onto the heel of each hind paw through the glass plate. The light beam was turned off when the rat lifted the foot, allowing measurement of PWL, which was defined as the time between when the light beam hit the foot and when the foot was lifted. Each trial was performed in triplicate at 5-minute intervals. A cut-off time of 20 seconds was imposed to avoid tissue injury.

Drug Treatment in Model of Acute Inflammatory Pain

Thirty minutes after drug treatment, acute inflammatory pain was induced by subcutaneous injection of 50 µl of 5% formaldehyde into the hind paw dorsum as described by Snijdelaar et al and our previous reports (24,25). This produced a typical flinching and biting response comprising 2 phases: an initial quiescent phase 1 and a prolonged tonic phase 2 beginning about 10 minutes after injection. Duration of biting and elevating the leg was recorded in 5-minute intervals until 60 minutes after injection. The pain score was calculated using the following equation (26):

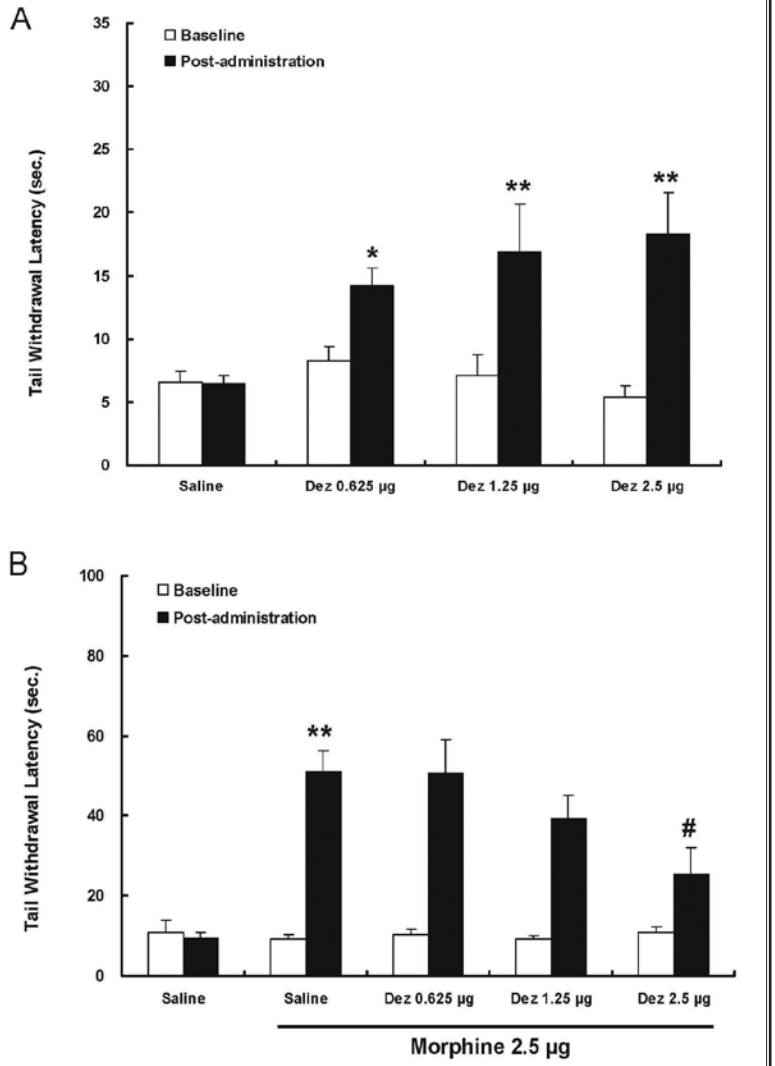
Pain score = [Duration of elevation + (2 × Duration of biting)] / 300.

Assessments

Analysis of ERK Phosphorylation Following Plantar Incision

The phosphorylation of ERK in the spine was determined by Western blot as in our previous reports (27,28). Rats in the acute incision model were treated with saline or drugs as described above, then deeply anesthetized by intraperitoneal injection with pentobarbital sodium (100 mg/kg; Sinopharm Chemical Reagent Co., Ltd, Shanghai, P. R. China) and decapitated. The spinal lumbar enlargement was quickly excised, and divided into ipsilateral and contralateral halves relative to the axis of incision. The ipsilateral half was further divided into dorsal and ventral quadrants. The ipsilateral dorsal quadrants were homogenized in ice-cold homogenization buffer (Beyotime Institute of Biotechnology, Haimen, P. R. China). Homogenates were centrifuged at 10,000 × g for 10 minutes at 4°C, the supernatant was collected, and total protein concentration was determined using the Micro BCA Pro-

tein Assay Reagent Kit (Thermo Fisher Scientific, Waltham, MA). Equal amounts of total protein were separated using 8% sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and then transferred to a polyvinylidene difluoride membrane using a wet transfer apparatus. Proteins bound to the membrane were stained with Ponceau S solution (Sigma-Aldrich, St Louis, MO) to determine the quality of the transfer. Membranes were blocked for 2 hours at room temperature and then incubated overnight at 4°C with primary antibody against pERK (1:2000; Upstate Biotechnology, Lake Placid, NY), ERK (1:6000; Upstate), and β-tubulin (1:2000; Beyotime). The incubation buffer contained 50 µl Tween-20 per 100 mL of buffer. After washing with Tris-buffered saline containing Tween-20, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:2000; Proteintech Group, Chicago, IL) for 2 hours at room temperature. Finally, membranes were washed thoroughly and protein bands were visualized using the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific), captured using the ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA) and quantified using Quantity One 4.62 software (Bio-Rad Laboratories).



*P < 0.05, **P < 0.01 vs saline; #P < 0.05 vs morphine and saline. Values are based on 12 animals, and error bars indicate SEM.

Fig. 1. *Analgesic effects of dezocine and antagonism of morphine analgesia in the tail flick test. After baseline tail withdrawal latency was determined, mice were treated with 0.625 – 2.5 µg dezocine (Dez) alone or in combination with 2.5 µg morphine per animal and then latency was re-determined 30 minutes later. (A) Dezocine increased tail withdrawal latency in a dose-dependent fashion at 30 minutes after intrathecal injection. (B) Dezocine antagonized the analgesic effect of morphine in a dose-dependent fashion.*

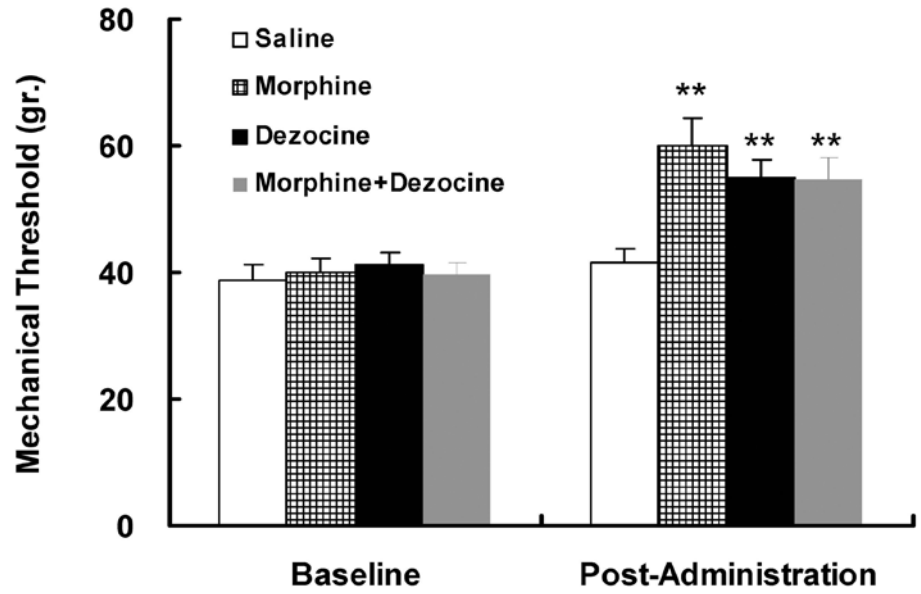
Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) and analyzed by one-way or 2-way analysis of variance (ANOVA), followed by the least significant difference test for multiple comparisons. Non-parametric analyses were performed using the Mann-Whitney test. P < 0.05 was defined as the threshold of statistical significance.

Results

Analgesic Effects of Dezocine Administered Alone or with Morphine in the Tail Flick Test

Administering dezocine alone to mice in doses of 0.625 – 2.5 µg per animal led to significantly longer TWL than saline when assessed at 30 minutes after intrathecal injection, and the increase in TWL was dose-dependent (F = 4.159, ANOVA = 0.011, P < 0.01 or 0.05, n = 12, Fig. 1A). The same dose range of dezocine antagonized the analgesic effect of co-administered morphine (2.5 µg) in a dose-dependent fashion (F = 8.669, ANOVA = 0.000, P < 0.05, n = 12, Fig. 1B).



**$P < 0.01$ vs saline group. Values are based on 8 animals, and error bars indicate SEM.

Fig. 2. *Dezocine reduces morphine analgesia in uninjured rats. After a baseline von Frey test was performed, rats were treated with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (both at 3 mg/kg) and then subjected to the von Frey test 60 minutes later. Administration of morphine or dezocine alone led to a significantly higher mechanical threshold than administration of saline. Co-administration of both drugs did not further increase the threshold.*

Dezocine Antagonism of Morphine Analgesia in Physiological Nociception

Rats were treated with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (3 mg/kg morphine and 3 mg/kg dezocine) and then MT was measured one hour later. MT was significantly higher in all these groups than in control animals treated with saline (F = 5.662, ANOVA = 0.004, $P < 0.01$, n = 8, Fig. 2). Either morphine or dezocine alone led to similar MT and the combination of both (morphine 3 mg/kg and dezocine 3 mg/kg) led to slightly but not significantly lower MT.

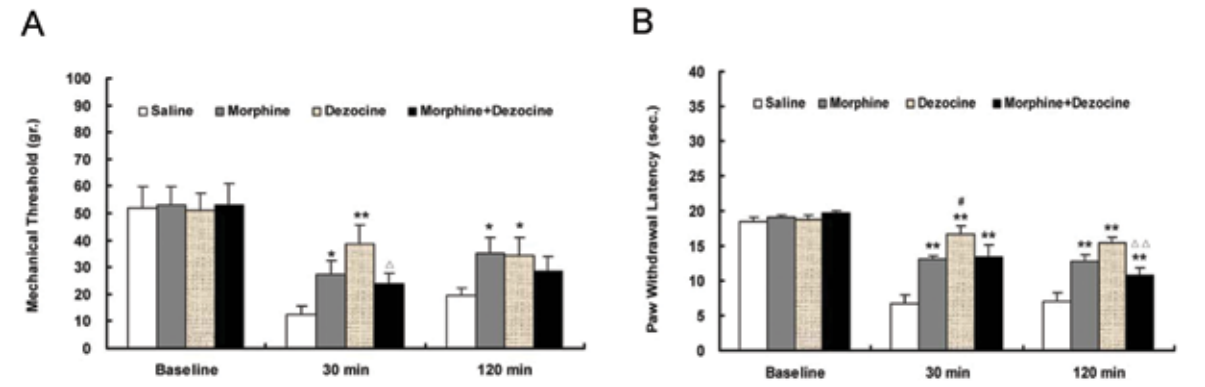
Dezocine Antagonism of Morphine Analgesia in Acute Incision Pain

The validity of the incision pain model was confirmed by showing that plantar incision significantly decreased MT and PWL in rats treated with saline (Fig. 3A and B). Treatment with morphine (3 mg/kg) or dezocine (3 mg/kg) led to significantly higher MT than control treatment with saline at 30 minutes (F = 5.226, ANOVA = 0.005, $P < 0.01$ or 0.05, n = 8, Fig. 3A) and 120 minutes ($P < 0.05$, n = 8, Fig. 3A) after incision. Dezocine led to a slightly but not significantly higher MT at 30 minutes than morphine. Treatment with both drugs together (both at 3 mg/kg) reduced the MT to the similar level as morphine, which is lower than dezocine alone at 30 minutes ($P < 0.05$, n = 8, Fig. 3A).

Treatment with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (both at 3 mg/kg) led to significantly higher PWL than control treatment with saline at 30 minutes (F = 12.724, ANOVA = 0.000, $P < 0.01$, n = 8, Fig. 3B) and 120 minutes after incision (F = 12.060, ANOVA = 0.000, $P < 0.01$, n = 8, Fig. 3B). Dezocine led to a significantly higher PWL than morphine at 30 minutes ($P < 0.05$; n = 8, Fig. 3B) and slightly but not significantly higher PWL at 120 minutes. However, co-administration of both drugs led to a significantly lower PWL at 120 minutes after incision than administration of dezocine alone ($P < 0.01$; n = 8, Fig. 3B).

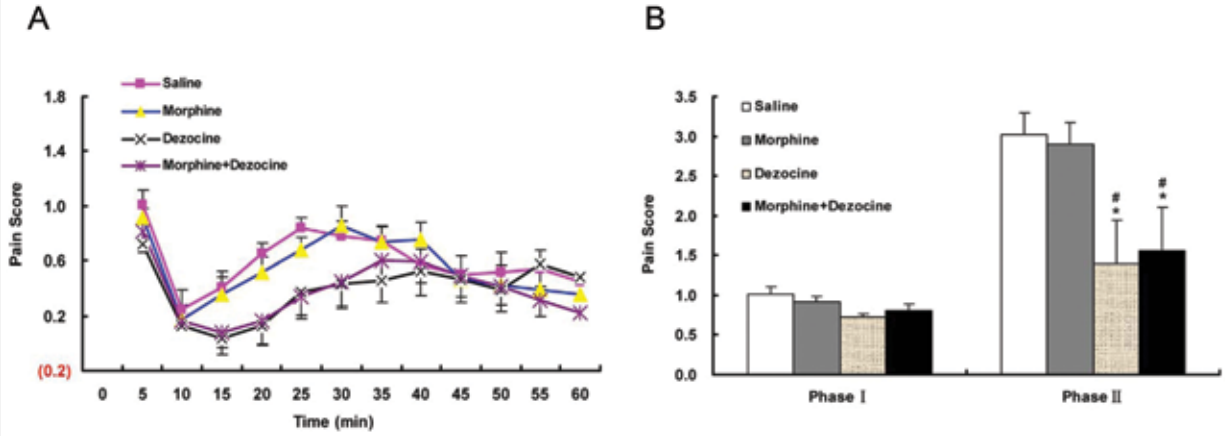
Analgesic Effects of Dezocine on Formaldehyde-induced Acute Inflammatory Pain

Rats exhibited biphasic nociception after intraplantar formaldehyde injection, with phase 1 occurring



**$P < 0.01$, *$P < 0.05$ vs saline group; #$P < 0.05$ vs morphine; $^{\Delta\Delta}P < 0.01$, $^{\Delta}P < 0.05$ vs dezocine. n = 8. Error bars indicate SEM.

Fig. 3. *Dezocine reduces morphine analgesia in acute incision pain. Rats were treated with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (both at 3 mg/kg) and then subjected to plantar incision on the right hind paw. At 30 and 120 minutes, animals were subjected to a von Frey test to measure mechanical threshold or to thermal stimulation to measure paw withdrawal latency. Mechanical threshold at 30 minutes and 120 minutes were significantly higher in animals treated with either drug alone than in animals treated with saline, whereas the combination of both drugs led to similar results as morphine, which is lower than dezocine alone at 30 minutes (A). Morphine or dezocine alone or with both in combination led to higher withdrawal latency at 30 minutes and 120 minutes than saline. Dezocine produced higher withdrawal latency at 30 minutes than morphine. But the combination of dezocine with morphine led to a lower latency at 120 minutes than dezocine alone (B).*



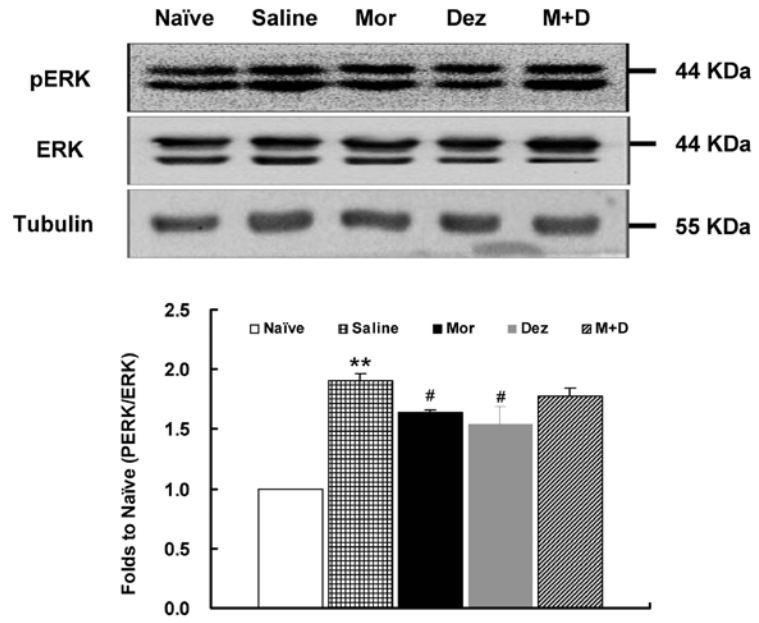
*$P < 0.05$ vs saline group; #$P < 0.05$ vs morphine group. Values are based on 8 animals, and error bars indicate SEM.

Fig. 4. *Analgesic effects of dezocine on formaldehyde-induced acute biphasic nociception. Rats were treated with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (both at 3 mg/kg) and then acute nociception was induced by intraplantar formaldehyde injection. (A) Pre-administration with 3 mg/kg morphine did not inhibit the biphasic reaction, while 3 mg/kg dezocine reduced the pain score in phase 2. The combination of both 3 mg/kg dezocine and 3 mg/kg morphine did not further inhibit formaldehyde-induced nociception. (B) Phase 2 pain scores were significantly lower in animals treated with dezocine alone or the combination of both drugs than in control animals treated with saline.*

at 0 – 5 minutes and phase 2 at 20 – 60 minutes (Fig. 4A). Pretreating the animals with morphine (3 mg/kg) did not inhibit the biphasic reaction, while pretreating them with dezocine (3 mg/kg) significantly reduced the pain score in phase 2 ($F = 3.929$, ANOVA = 0.019, $P < 0.05$, n = 8, Fig. 4A and B). Pretreatment with both drugs together led to similar results as pretreatment with dezocine alone.

Effects of Dezocine on Incision-induced Phosphorylation of ERK in the Ipsilateral Dorsal Horn

Plantar incision of rats significantly induced the expression of pERK in the ipsilateral spinal dorsal horn ($F = 12.115$, ANOVA = 0.001, $P < 0.01$, Fig. 5). Pretreatment with either dezocine or morphine alone, but not the combination of these 2 drugs, significantly inhibited the incision-induced phosphorylation of spinal pERK ($P < 0.05$).



Mor = morphine + incision; Dez = dezocine + incision; M + D = morphine + dezocine + incision. $^{**}P < 0.01$ vs naïve group; $\#P < 0.05$ vs saline group. Values are based on 4 animals, and error bars indicate SEM.

Fig. 5. *Dezocine-mediated inhibition of ERK phosphorylation in the spinal dorsal horn following plantar incision. Rats were treated with morphine (3 mg/kg), dezocine (3 mg/kg), or both drugs together (both at 3 mg/kg) and then subjected to plantar incision on the right hind paw. After 120 minutes, animals were decapitated and the phosphorylation of ERK in the ipsilateral spinal dorsal horn was detected. Incision induced the phosphorylation of spinal pERK, and this induction was inhibited by pretreatment with either dezocine or morphine alone, but not the combination of both drugs. Groups are labeled as follows: Naive, animals were administered saline instead of drug and received no incision; Saline, animals were administered saline and then given an incision.*

Discussion

Dezocine, a weak opioid analgesic is widely used to manage perioperative pain in China and other Asian countries. It exerts both agonistic and antagonistic actions on opioid receptors (7,29), thus leading it to interact in complex ways with other analgesics like morphine (Table 1). Here we used rat and mouse models of multiple nociception to help clarify the overall effects of dezocine when used alone and in combination with morphine. In all 3 contexts, our results suggest that dezocine antagonizes the analgesic effects of morphine when the 2 drugs are co-administered.

Intrathecal administration of dezocine alone increased the pain threshold to nociceptive heat stimulation while co-administration of dezocine and morphine attenuated the latter's analgesic effects; both effects depended on dezocine dose. Intraperitoneal administration of dezocine alone increased the pain threshold to mechanical stimulation in uninjured rats as much as administration of morphine alone, as proven by reports in humans (11). The combination of drugs did not show any synergic or additive effect in pain threshold. In a model of acute incision pain, dezocine produced a more potent analgesia than morphine. However, using dezocine and morphine together did not elicit a synergistic effect but rather a level obtained with morphine alone.

These results obtained in various acute pain contexts suggest that dezocine antagonizes the analgesic effects of morphine. To our knowledge, this is the first report indicating that dezocine can show strong analgesic effects on its own whereas it antagonizes morphine's effects when the 2 drugs are co-administered. This may reflect the recent discovery that dezocine is a partial μ-receptor agonist and κ-receptor antagonist; it is totally different from the structurally similar pentazocine, which is a μ-receptor antagonist and κ-receptor agonist (7).

This discovery helps explain our results in the animal model of formaldehyde-induced acute inflammatory nociception. In those experiments, morphine at 3 mg/kg failed to inhibit either phase 1 or 2 of the pain response. This is congruent with previous reports showing that morphine doses of 6 – 7 mg/kg are required to inhibit formaldehyde nociception

Table 1. *Pharmacokinetic profiles of morphine and dezocine.*

Pharmacokinetic parameters	Morphine	Dezocine
Plasma protein binding %	30 - 40	NA
Peak level	15 – 30 min (IV) 45 – 90 min (IM/SC) 60 min (PO)	0.5 – 2h (IM)
Bioavailability (%)	40 – 50 (PO)	97 (IM)
Volume of distribution (l/kg)	1 to 6	NA
Plasma half-life ($t_{1/2}$)	2 – 4h (PO)	2.2h (IM) 1.7 – 2.6h (5mg IV) 2.4 – 2.6h (10mg IV)
Metabolizing organ	Liver 90% Gastrointestinal tract	Mainly liver (Glucuronidation/Sulfate formation)
Phase I metabolism	CYP3A	NA
Phase II metabolism	UGT2B7	NA
Major metabolites	M3G (50%) M6G (10%)	NA
Elimination	Renal (87%) Biliary excretion 7 - 10%	Mainly renal

NA, not applicable; IV, intravenous; IM, intramuscular; SC, subcutaneous; PO, orally; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide.

(30-32). In contrast, dezocine at 3 mg/kg inhibited the phase 2 pain response, presumably due to inactivation of spinal κ-opioid receptors. Phase 2 of the acute inflammatory pain response is thought to involve sensitization of dorsal horn neurons and peripheral neurons (33,34). Therefore, our findings in an animal model of formaldehyde-induced acute inflammation suggest that dezocine may exert a more potent pre-emptive analgesic effect than morphine, and we postulate that this is because dezocine inactivates spinal κ-receptors, whereas morphine is a pure μ-receptor agonist.

To provide preliminary molecular evidence that dezocine antagonizes morphine-mediated analgesia, we compared levels of pERK in animals treated with either drug alone or both drugs together. The animal models were then subjected to acute incision pain. Higher levels of pERK in the spinal dorsal horn correlate with greater neuronal activation in nociceptive pathways. Levels were significantly lower in animals treated with either the dezocine or morphine alone than in animals treated with saline. Animals treated with the combination of both drugs showed levels similar to those of saline controls. These molecular results are consistent with our behavioral findings suggesting that co-administration of both drugs inhibits morphine's analgesic effects.

Our series of animal studies provided evidence that pre-emptive dezocine administration is at least as effective as pre-emptive morphine administration for treating acute pain, while the combination of both drugs does not enhance the analgesic effect of either one. These results provide a basis for well-designed clinical trials to verify that dezocine can serve as an alternative to morphine with less of an addictive effect and less of a depressive effect on respiratory function. Trials should also verify whether using both drugs together to manage perioperative pain can sometimes induce hyperalgesia, resulting in higher opioid consumption with no additional clinical benefit.

Conclusion

We found that dezocine antagonizes morphine analgesia on acute nociception upon simultaneous administration.

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