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Dextromethorphan Potentiates Morphine-Induced Antinociception at both Spinal and Supraspinal Sites but Is Not Related to the Descending Serotoninergic or Adrenergic Pathways

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Key Words
Adrenergic pathway · Antinociception · Dextromethorphan · Morphine · Serotoninergic pathway

Abstract
Morphine is a strong and widely used opioid analgesic in pain management, but some adverse effects limit its clinical use at high doses. The clinically available non-opioid antitussive, dextromethorphan (DM) can potentiate the analgesic effect of morphine and decrease the dose of morphine in acute postoperative pain. However, the mechanism underlying this synergistic phenomenon is still not clear. To examine if the potentiation by DM occurs through the descending pain-inhibitory pathways, ketanserin (a 5-HT_2 receptor antagonist) and yohimbine (an α_2-adrenergic receptor antagonist) were employed and found to have no significant effect on the potentiation by DM. Using local delivery of drugs in rats in the present study, potentiation of morphine-induced antinociception by DM was observed via both intrathecal and intracerebroventricular routes, suggesting that both spinal and supraspinal sites are involved. This suggests that the potentiation of morphine-induced antinociception by DM is not mediated by the serotoninergic or adrenergic descending pain-inhibitory pathways. The present results are consistent with findings in clinical studies, which showed that DM can effectively decrease the consumption of morphine in patients suffering from pain. Since DM has excellent clinical potential as a synergistic agent with morphine, further investigating and clarifying the possible pharmacological mechanism of DM are of great importance for future studies.

Introduction

Morphine has been administered for over 100 years to allay anxiety and is widely used to reduce pain associated with disease and surgery. It is the most important, strong opioid an algesic presently available and is recommended by the World Health Organization as the drug of choice for the treatment of moderate to severe, acute or chronic, postoperative, and cancer pain. Some adverse effects, however, such as nausea, vomiting, hypotension, constipation, and respiratory depression, etc., have limited its clinical use at high doses. Chronic administration of mor-

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phine leads to tolerance to its analgesic effects, and increasing the drug dose to maintain analgesia leads to the appearance of undesirable side effects [40, 44]. Recent findings from animal studies have suggested that N-methyl-*D*-aspartate (NMDA) receptor antagonists, such as MK-801 [15, 34] or ketamine [2, 32], can potentiate the antinociceptive effects of morphine and decrease the dose of morphine [53]. Indeed, ketamine has been suggested to work as a useful adjunct with morphine in the treatment of pain [55]. But its narrow therapeutic window, short duration of action, and severe side effects such as hallucinations and psychotomimetic side effects have limited its role in acute pain management [50]. Severe neurotoxicities from MK-801 have also limited its use in clinical trials.

Another clinically available NMDA receptor antagonist is dextromethorphan (DM), which is a well-known over-the-counter antitussive with an established safety record of about 50 years [4, 38]. The therapeutic cough-suppressant dose of DM (1 mg/kg/day) produces no major opioid-like analgesia, respiration-depressing, or hemodynamic side effects, nor does it induce complications due to histamine release [5]. In a previous clinical double-blind study [6], we found that the preoperative administration of intravenous DM significantly reduced the postoperative morphine consumption for 48 h. Grass et al. [15] and Hoffmann et al. [19] also showed that systemically administered (intraperitoneally or subcutaneously) DM can potentiate the antinociceptive effect of morphine rather than reverse its tolerance in rats. Weinbroum et al. [49] reviewed and described how DM attenuated the sensation of acute pain at oral doses of 30–90 mg without significant side effects, and reduced the amount of analgesics in 73% of DM-treated patients postoperatively. Therefore, DM seems to be a useful synergistic agent with morphine for the treatment of pain. However, to our knowledge, there are few specific studies which have focused on investigating the mechanism underlying the potentiation of morphine-induced antinociception by DM.

Our preliminary experiments showed that DM can affect the concentration of morphine in the plasma of rats [data not shown, manuscript in prep.]. Therefore, in order to avoid the interference by pharmacokinetic factors, animals were treated with drugs localized at the spinal or supraspinal level, and examined for the antinociceptive effects by the tail-flick test in the present study. We hoped that these experiments would clarify if the action of DM in potentiating morphine-induced antinociception is exerted at the spinal or supraspinal level. To further investigate the possible mechanism of DM, a selective α_2 antagonist (yohimbine) and a 5-HT_2 antagonist (ketanserin) were used to block the descending pain-inhibitory pathways which have been reported to mediate the supraspinal antinociceptive action of systemically administered morphine [41, 54]. Much evidence indicates that morphine, probably by disinhibition of GABAergic neurons, can activate two major descending pain-inhibitory pathways, a serotoninergic and an adrenergic pathway from the raphe nucleus and the locus ceruleus, respectively [21, 35]. Therefore, potentiation of morphine-induced antinociception by DM may be related to the enhancement of one or two of these neuropathways, and so this was examined in the present study.

At the molecular level, DM has been reported to be capable of binding to NMDA receptors (at the phencyclidine site) [11, 28], nicotinic receptors [17], and sigma receptors [7]. Although DM is generally recognized as having a role as a weak NMDA antagonist, the possible effect of DM on morphine binding affinities to μ-opioid receptors has rarely been formally examined. Using a [^{3}H]DAMGO binding assay, DM was tested for its possible effect on morphine binding to μ-opioid receptors in the present study. These experimental results should be helpful in clarifying whether the potentiation of morphine-induced antinociception by DM occurs at the μ-opioid receptor level.

Materials and Methods

Experimental Animals

The experimental protocol was approved by the Animal Care and Use Committee of the National Defense Medical Center, Taiwan. Ten-week-old male Sprague-Dawley rats weighing between 300 and 350 g (purchased from the National Experimental Animal Center, Taipei, Taiwan) were used. Three rats were kept in a cage before surgery, but they were housed 1 rat per cage after surgery. All animals were kept in the animal rooms of the National Defense Medical Center before the experiments. The animal rooms were maintained at 23 ± 2 °C with a 12-hour light/dark cycle. Food and water were available ad libitum throughout the experiment.

Chemicals

[^{3}H]DAMGO was purchased from Perkin-Elmer Life Sciences (Boston, Mass., USA). Morphine hydrochloride was purchased from the National Bureau of Controlled Drugs, National Health Administration, Taipei, Taiwan, ROC. Dextromethorphan and MK-801 were purchased from RBI (Natick, Mass., USA). All of the other chemicals were supplied by Sigma (St. Louis, Mo., USA).

Determination of the Antinociceptive Effects of the Drugs

Morphine- or drug-induced antinociception was evaluated by the tail-flick test [10, 43]. Basal latency was first determined and found to

range from 2.5 to 3.5 s. The cutoff time was set at 10 s to prevent excess injury to the tail. For each rat, the basal latency was determined at least three times, and then the drug to be tested was injected. Tail-flick latency was recorded at 30, 60, 90, 120, 150, 180, 210, and 240 min after drug administration. The antinociceptive response is presented as the tail-flick latency minus the basal latency as shown in figure 1A. The area under the time-response curve (AUC) as shown in figure 1B was calculated following the trapezoidal rule [16] using computer programs of pharmacologic calculations (PHARM/PCS, Springer, New York, N.Y., USA). The AUC value was regarded as an index of the antinociceptive effect of the drugs.

Implantation of an Intrathecal Catheter and an Intracerebroventricular Guide Cannula

For implantation of intrathecal catheters, rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and an intrathecal catheter was implanted at the lumbar level for drug administration as previously described [52]. For implantation of an intracerebroventricular cannula, animals were first anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then a stainless-steel cannula (thin-walled, 23-gauge stainless-steel tube) was implanted into the left lateral ventricle according to the coordinates: p +1.0 mm, L +1.25 mm, and V −4 to 5 mm using the bregma as the origin [31, 41]. The cannula was firmly attached to the skull with two metal screws and dental cement [41]. Each animal was allowed 4 days to recover from the surgery, and was not used for more than one experiment. Any rat showing motor impairment was not employed in the following study.

One day before the experiment, animals with intrathecal catheters were injected with 20 µl of 2% lidocaine in a microsyringe (Hamilton, 25 µl) to induce 10–20 min of temporary motor blockade of the lower limbs if the catheter was in the correct position. For animals with the intracerebroventricular guide cannula, the position of the cannula was confirmed by negative pressure from the lateral ventricle, which caused 5 µl of saline in the catheter connected to the cannula to fall freely by gravity.

Drug Administration

In the experiments using systemic administration, drugs were administered subcutaneously (s.c.), and the rats were randomly divided into four groups: saline (control), morphine (M), dextromethorphan (DM), and dextromethorphan+morphine (DM+M). There were at least 6 rats in each group.

For local administration of drugs, intrathecal or intracerebroventricular injections were given to animals. In order to evaluate the impact of intrathecal yohimbine (Yoh) on the ability of DM to potentiate the antinociceptive effect of morphine, rats were randomly divided into four groups in another set of experiments. Both the morphine and DM+M groups were intrathecally (i.t.) pretreated with saline 20 min prior to drug injection. Afterwards, 0.5 µg morphine (i.t.) or 0.5 µg morphine (i.t.) co-administered with 2 µg DM was given to the morphine or DM+M group, respectively. Both the Yoh+M or Yoh+DM+M groups were pretreated with 15 µg yohimbine (i.t.) 20 min before the injection of intrathecal morphine or intrathecal morphine co-administered with intrathecal DM, respectively. The effect of pretreatment with ketanserin was also tested in the same manner. Rats were divided into the morphine, DM+M, Ket+M, and Ket+DM+M groups, but the dose of ketanserin was 5 µg (i.t.).

At the supraspinal level, the effect of ketanserin or yohimbine pretreatment was also examined in a similar way as described above

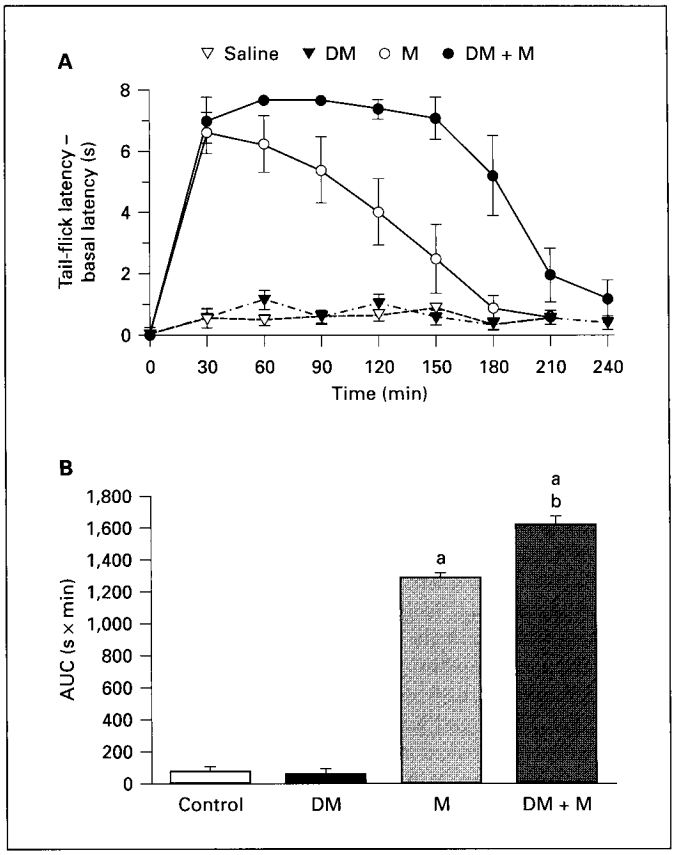

Fig. 1. The effect of systemic DM on the antinociceptive effect of morphine (M). **A** Tail-flick latency was measured every 30 min after subcutaneous injection of saline or drug(s). Basal latency (2.5–3.5 s) was determined before drug administration. **B** The AUC (shown in **A**) was calculated according to the trapezoidal rule. One-way ANOVA followed by the Newman-Keuls test was used to analyze the data. [a] $p < 0.01$ vs. the control group, [b] $p < 0.01$, vs. the M group. Means ± SEM. Each group contains at least 6 rats.

but via intracerebroventricular (i.c.v.) administration. The doses of morphine and DM were 2 (i.c.v.) and 8 µg (i.c.v.), respectively; the dose of yohimbine pretreatment was 15 µg (i.c.v.), and the dose of ketanserin pretreatment was 5 µg (i.c.v.). Both ketanserin and yohimbine pretreatments were performed 20 min prior to intracerebroventricular administration of morphine or DM+M.

In another set of experiments, morphine (2 µg, i.c.v.) or morphine (2 µg, i.c.v.) + DM (8 µg, i.c.v.) was given supraspinally, but the pretreatment with ketanserin (5 µg, i.t.) or yohimbine (15 µg, i.t.) was performed intrathecally. Following each intrathecal or intracerebroventricular injection, the catheter was gently flushed with 10 or 5 µl of saline, respectively.

Receptor Binding Assay

Opioid-naïve rats were decapitated, and their brains (excluding the cerebellum) were quickly frozen on dry ice, and weighed. Brain tissues were thawed and placed in 50 m*M* Tris buffer (pH 7.4;

1:10 w/v), homogenized by a Polytron setting at 6 for 20 s in a cooling ice bath, and centrifuged for 20 min (25,000 *g*, 4 °C) [27, 39]. The supernatant was discarded, and membranes were saved. Tris buffer was added (1:50 w/v), and the pellet was resuspended and placed in a 36.5 ± 0.5 °C water bath for 20 min to remove endogenous opioids. The homogenate was centrifuged at 25,000 *g* (4 °C) again for 20 min, and the supernatant was discarded. The entire homogenization and centrifugation process was repeated three times. The final pellet was resuspended in 3 ml of 50 m*M* Tris buffer, and stored at −70 °C.

The saturation binding assay of [^{3}H]DAMGO was first carried out to determine the K_D of [^{3}H]DAMGO with final concentrations of 0.1, 0.3, 0.5, 1.0, 3.0, 5.0, and 10.0 n*M*. Competition binding studies were next carried out to determine the K_i of morphine and also the effect of DM on the affinity of morphine to bind to the μ-opioid receptors. Different concentrations of morphine (final concentrations of 0.5, 1, 3, 5, 10, 30, and 50 n*M*) were added to Tris buffer containing 1.0 n*M* [^{3}H]DAMGO, 10 m*M* $MgCl_2$, and the membrane suspension. In order to examine the effect of DM on morphine binding, an identical set of solutions was prepared, and DM (at final concentrations of 2, 4, 12, 20, 40, 120, and 200 n*M*) was also included in the buffer for binding incubation. Nonspecific binding was determined by adding 1 μ*M* naloxone.

Binding reactions were allowed to proceed at room temperature for 2 h. Then, the incubation suspensions were filtered by a Skatron semi-automatic cell harvester (model 11025, Skatron Instruments, Sterling, Va., USA), and washed three times with Tris buffer. Filters were collected and soaked in scintillation cocktail (Ready Safe, Beckman, Fullerton, Calif., USA) overnight at room temperature. The radioactivity of the cocktail with the filters was measured in a β-counter (LS-6000TA, Beckman). Protein concentrations of the membranes were assayed by the Lowry method [25]. B_{max}, K_D, and K_i were calculated using the EBDA and Ligand programs [42].

Statistical Analyses

Results are all expressed as the mean ± SEM. Analysis of variance (ANOVA) was used to assess the statistical significance of the repeated measures of the overall data, and differences between the individual mean values in different groups were analyzed by the Newman-Keuls test. For comparison of two different groups, unpaired t test was used. Differences were considered to be significant at $p < 0.05$.

Results

Effect of Systemically Administered DM on Morphine-Induced Antinociception

The tail-flick latencies of rats in the saline (control) and DM groups were quite stable over time (fig. 1A). There was no antinociceptive effect induced by saline or DM (20 mg/kg, s.c.). The antinociceptive effects of morphine (5 mg/kg, s.c.) or DM (20 mg/kg, s.c.) + morphine reached a maximum at 30 min and then gradually diminished between 180 and 210 min after the injection. The duration of the antinociceptive effect of morphine was prolonged by DM, but the efficacy was not changed (fig. 1A). As indicated by the AUC of the tail-flick response over time (fig. 1B), morphine induced significant antinociception (1,284.0 ± 35.8 vs. 69.6 ± 28.5 s × min), and DM further potentiated morphine-induced antinociception from 1,284.0 ± 35.8 to 1,618.3 ± 53.0 s × min (a 26.0% increase).

Effect of Intrathecal Pretreatment with Ketanserin or Yohimbine on the Ability of DM to Potentiate the Antinociception of Morphine at the Spinal Level

As shown in figure 2, we found that intrathecal DM (2 μg, i.t.) also potentiated antinociception induced by morphine (0.5 μg, i.t.) to a similar extent (AUC: 816.4 ± 40.3 vs. 1,029.8 ± 48.5 s × min; a 26.1% increase) as that of the systemic route.

Intrathecal ketanserin pretreatment was found to cause a 16.5% decrease in the antinociceptive effect of morphine (the AUC decreased from 816.4 ± 40.3 to 681.8 ± 40.0 s × min; fig. 2). When 5 μg of ketanserin pretreatment was given intrathecally, DM was still able to increase morphine (0.5 μg, i.t.) antinociception from 681.8 ± 40.0 to 863.1 ± 36.2 s × min (a 26.6% increase; fig. 2).

Similarly, intrathecal yohimbine pretreatment (15 μg) also caused a decrease (17.7%) in the antinociceptive effect of morphine from 917.0 ± 46.4 to 754.9 ± 45.9 s × min (fig. 3). However, DM was still able to potentiate morphine (0.5 μg, i.t.) antinociception as well under this condition (AUC: 986.8 ± 42.8 vs. 754.9 ± 45.9 s × min for Yoh+DM+M vs. Yoh+M, respectively; a 30.7% increase as shown in fig. 3).

Effect of Intracerebroventricular Pretreatment with Ketanserin or Yohimbine on the Ability of DM to Potentiate Morphine Antinociception at the Supraspinal Level

When DM (8 μg, i.c.v.) was given supraspinally, it also significantly potentiated the antinociceptive effect of supraspinal morphine (2 μg, i.c.v.) from 778.4 ± 56.1 to 1,094.6 ± 65.7 s × min (fig. 4). The increase in AUC was 40.6%.

Supraspinal pretreatment with ketanserin (5 μg, i.c.v.) decreased morphine antinociception by 33.4% (fig. 4). When ketanserin was pretreated 20 min before the injection of morphine (2 μg, i.c.v.) or DM+M (8 μg of DM + 2 μg of morphine, i.c.v.) via the intracerebroventricular route, DM still potentiated morphine antinociception by 41.3% (fig. 4).

Similarly, intracerebroventricular yohimbine (15 μg, i.c.v.) decreased morphine antinociception by 19.5%

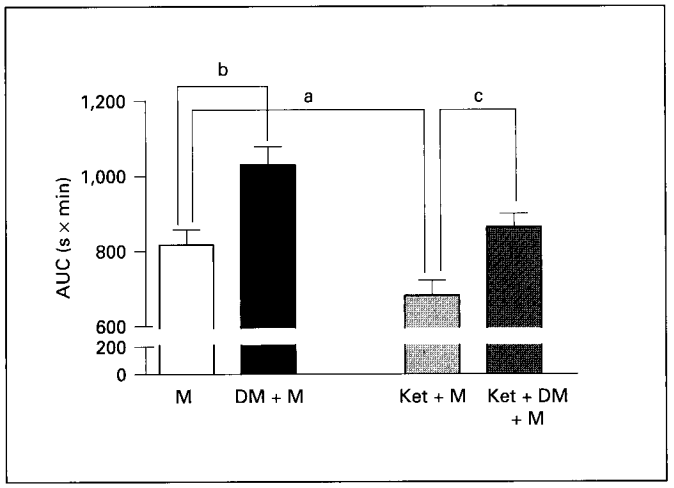

Fig. 2. The effect of intrathecal ketanserin (Ket, 5 μg) on the antinociceptive effect of morphine (M, 0.5 μg, i.t.) and the potentiating effect of DM (2 μg, i.t.) at spinal level. Means ± SEM. Each group contains at least 6 rats. [a] $p < 0.05$ and [b] $p < 0.01$, vs. the M group; [c] $p < 0.05$ vs. the Ket+M group.

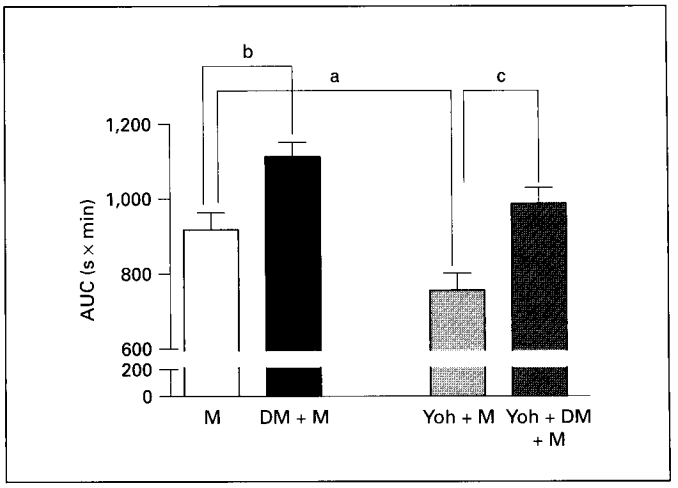

Fig. 3. The effect of intrathecal yohimbine (Yoh, 15 μg) on the antinociceptive effect of morphine (M, 0.5 μg, i.t.) and the potentiating effect of DM (2 μg, i.t.) at spinal level. Means ± SEM. Each group contains at least 6 rats. [a] $p < 0.05$ and [b] $p < 0.01$, vs. the M group. [c] $p < 0.01$ vs. the Yoh+M group.

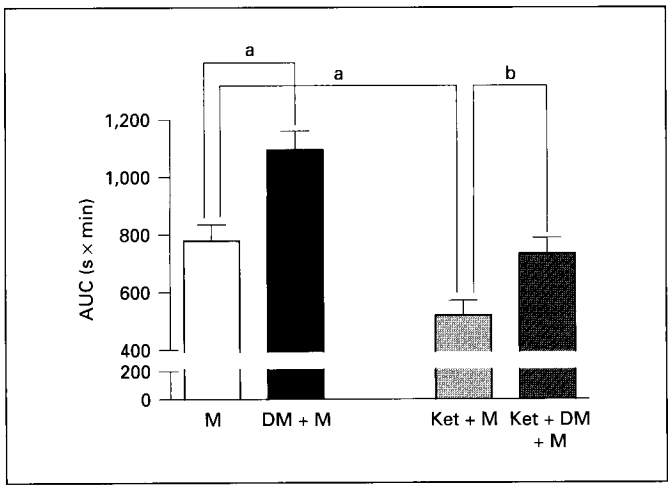

Fig. 4. The effect of ketanserin (Ket, 5 μg, i.c.v.) on the antinociceptive effect of morphine (M, 2 μg, i.c.v.) and the potentiating effect of DM (8 μg, i.c.v.) at supraspinal level. Each group contains at least 6 rats. [a] $p < 0.01$ vs. the M group, [b] $p < 0.01$ vs. the Ket+M group.

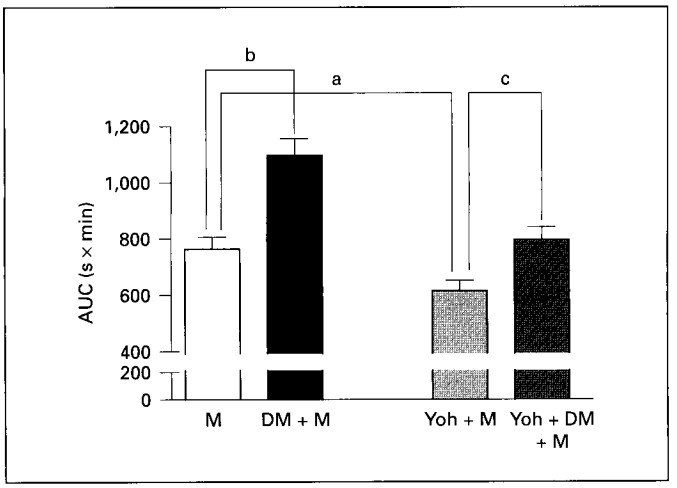

Fig. 5. The effect of yohimbine (Yoh, 15 μg, i.c.v.) on the antinociceptive effect of morphine (M, 2 μg, i.c.v.) and the potentiating effect of DM (8 μg, i.c.v.) at supraspinal level. Each group contains at least 6 rats. [a] $p < 0.05$ and [b] $p < 0.01$, vs. the M group, [c] $p < 0.05$ vs. the Yoh+M group.

(fig. 5). When yohimbine was given 20 min before the injection of morphine (2 μg, i.c.v.) or DM+M (8 μg of DM +2 μg of morphine, i.c.v.) via the intracerebroventricular route, DM still potentiated morphine antinociception by 29.4% (fig. 5).

Effect of Intrathecal Pretreatment with Ketanserin or Yohimbine on the Ability of DM to Potentiate Morphine Antinociception at the Supraspinal Level

As shown in figure 6, intrathecal ketanserin (5 μg, i.t.) decreased the antinociception induced by supraspinal morphine administration (2 μg, i.c.v.) by 19.1%. When

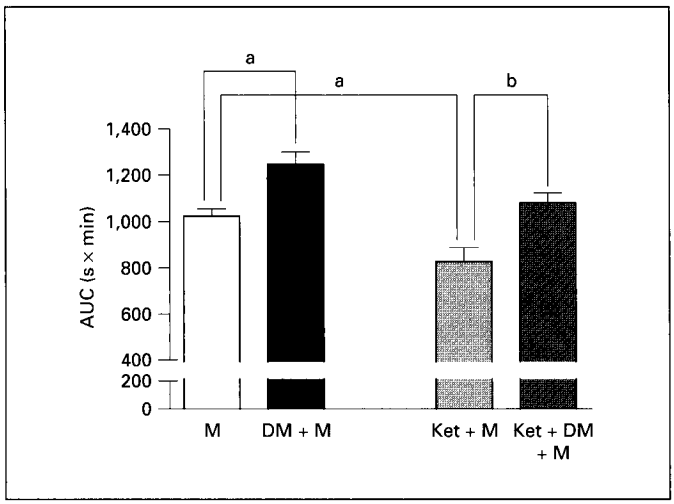

Fig. 6. The effect of intrathecal ketanserin (Ket, 5 μg, i.t.) on the supraspinal antinociceptive effect of morphine (M, 2 μg, i.c.v.) and the potentiating effect of DM (8 μg, i.c.v.). Each group contains at least 6 rats. [a] $p < 0.05$ vs. the M group, [b] $p < 0.01$ vs. the Ket+M group.

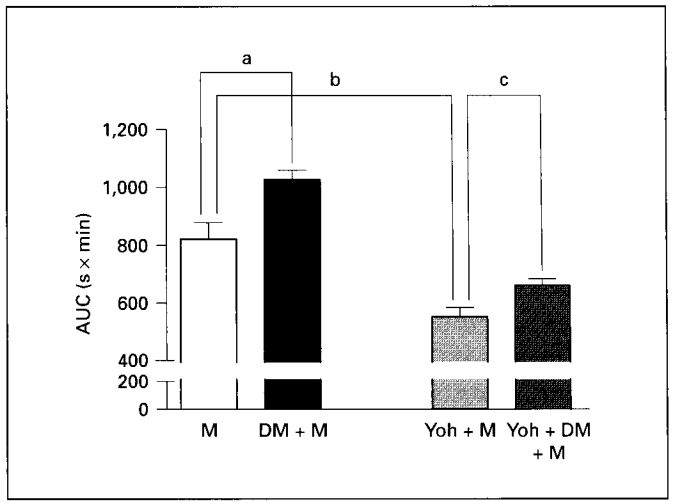

Fig. 7. The effect of intrathecal yohimbine (Yoh, 15 μg, i.t.) on the supraspinal antinociceptive effect of morphine (M, 2 μg, i.c.v.) and the potentiating effect of DM (8 μg, i.c.v.). Each group contains at least 6 rats. [a] $p < 0.05$ and [b] $p < 0.01$, vs. the M group, [c] $p < 0.05$ vs. the Yoh+M group.

Table 1. The results of the morphine competition binding to μ-opioid receptors in [^{3}H]DAMGO binding assay in the presence or absence of DM using cerebral membranes of morphine-naïve rats

Group	n	K_i, n*M*	B_{max}, fmol/mg
Morphine	6	2.09 ± 0.36	258.4 ± 34.0
DM + M	6	2.07 ± 0.25	253.6 ± 37.3

ketanserin (5 μg, i.t.) pretreatment was performed intrathecally, the potentiating effect of DM (8 μg, i.c.v.) on morphine (2 μg, i.c.v.) antinociception at the supraspinal level was still significant (a 30.6% increase; fig. 6).

Similarly, intrathecal yohimbine (15 μg, i.t.) also decreased the antinociception induced by intracerebroventricular morphine (2 μg, i.c.v.) by 35.2%. When yohimbine (15 μg, i.t.) pretreatment was performed intrathecally, the potentiating effect of DM (8 μg, i.c.v.) on morphine (2 μg, i.c.v.) antinociception at the supraspinal level was still significant (a 24.1% increase; fig. 7).

Effect of DM on μ-Opioid Receptor Binding

Cerebral membranes from morphine-naïve rats were used to determine the binding affinity of [^{3}H]DAMGO to μ-opioid receptors. The K_D value of [^{3}H]DAMGO was found to be 1.11 ± 0.14 n*M*. As shown in table 1, the K_i value for morphine binding to μ-opioid receptors was 2.09 ± 0.36 n*M* in the competition binding assay using [^{3}H]DAMGO. In the presence of DM (at 4 times the concentration of morphine), the K_i value for morphine binding to μ-opioid receptors was not significantly changed (K_i = 2.07 ± 0.25 n*M*). The B_{max} value of [^{3}H]DAMGO binding also showed no significant difference between the value obtained in the presence of morphine alone (258.4 ± 34.0 fmol/mg) and the one in the presence of both morphine and DM (253.6 ± 37.3 fmol/mg; table 1).

Discussion

In the present study, DM was demonstrated to potentiate morphine antinociception systemically (s.c.), which is consistent with other reports [15, 19]. In a further effort to determine the site(s) of action of DM, intrathecal and intracerebroventricular routes were also used to administer DM, and we found that DM (intrathecally and intracerebroventricularly) produced a similar extent of increase on morphine antinociception to that obtained from systemic DM. These results indicated that DM can potentiate morphine antinociception at both the spinal and supraspinal levels.

Among the descending pain-inhibitory pathways, the serotoninergic and adrenergic pathways are most important and well documented [37, 41]. Projecting from the nucleus raphe-magnus, the serotoninergic pathway terminates at the dorsal horn of the spinal cord, thereby reducing spinal nociception. Another spinal projection arises from the adrenergic cell groups in the pons and medulla and from the nucleus paragigantocellularis, which also mediates descending pain inhibition. Both descending pathways receive inputs from the periaqueductal gray (PAG) region. At the spinal level, nociception can be attenuated by the local opioids, norepinephrine (NE) and serotonin, which are released from descending inhibitory noradrenergic and serotoninergic pathways [37]. The spinal administration of NE [33] and serotonin [47] induces antinociception in the rat.

Antinociception produced by the supraspinal injection of morphine is blocked by intrathecally administered antagonists of adrenergic receptors [51], whereas it is enhanced by the intrathecally administered drugs that block the reuptake of NE [24]. In addition, the profound analgesic effect of morphine, via microinjection into the PAG [13], is attenuated by prior depletion of NE stores in the spinal cord [30], or intrathecal yohimbine (α_2-adrenergic antagonist), but not by intrathecal prazosin (α_1-adrenergic antagonist) [45]. Intrathecal injection of the selective α_2-adrenergic agonist, clonidine, also leads to analgesia in humans [26]. The results mentioned above may be caused by increasing activation of descending adrenergic pathways via the locus ceruleus [1], by a direct inhibitory effect on neuronal firing at receptor sites in the substantia gelatinosa [22], or by a reduction in the release of substance P [23].

An intrathecal morphine injection can trigger an antinociceptive effect by directly binding to the spinal μ-opioid receptor. Serotoninergic fibers are most abundant in superficial zones (laminae I/II and IV–VI). Intrathecal serotonin can also cause antinociceptive effects by affecting enkephalin release from spinal interneurons [46, 48]. Enkephalin receptors are also colocalized with α_2-adrenergic receptors in projection neurons [12, 20]. Several studies have demonstrated mutual (generally synergistic) spinal antinociceptive properties of α_2-adrenergic and opioid agonists in both acute and chronic inflammatory and neuropathic pain [9, 18]. In the present study (fig. 2, 3), both intrathecal ketanserin and yohimbine significantly inhibited the antinociceptive effect of intrathecal morphine, further indicating that the role of the serotoninergic receptor and α_2-adrenergic receptor in regulating spinal opioid antinociceptive effects.

In the present study using intrathecal pretreatment of ketanserin (a 5-HT_2 receptor antagonist) or yohimbine (an α_2-adrenergic antagonist), DM showed the same extent of potentiation of morphine antinociception at the spinal level. This indicates that the potentiation of morphine antinociception by DM at the spinal level may be independent of the activity of the serotoninergic and adrenergic pathways.

In another set of experiments, we examined the effect of supraspinal ketanserin or yohimbine pretreatment on intracerebroventricular morphine-induced antinociception. Ketanserin or yohimbine by intracerebroventricular administration should be able to diffuse to the nuclei which drive the descending pain-inhibitory pathways, thereby reducing the activity of antinociception. The same results were obtained, and it was found that DM still increased morphine antinociception under either ketanserin or yohimbine pretreatment (i.c.v.).

Moreover, the effect of intrathecal ketanserin and yohimbine pretreatment on intracerebroventricular morphine-induced antinociception was also investigated in the present studies. It is well known that morphine acts at supraspinal sites (e.g. the PAG) to generate potent antinociceptive effects [45]. Similarly, blockade by ketanserin or yohimbine at the spinal terminals of the descending pain-inhibitory pathways did not affect the potentiation of supraspinal morphine antinociception by DM (i.c.v.). Taken together with previous results, the action of DM to potentiate morphine antinociception seems to be exerted at both the spinal and supraspinal levels, and it is not related to the descending serotoninergic and adrenergic pathways.

A limitation on our conclusions regarding 5-HT receptors is that we only used an antagonist that is selective for 5-HT_2 receptors. However, based on reports by others [3, 29, 36], we speculated that the 5-HT_2 receptor is the key type involved in morphine-induced antinociception. There are four major types of 5-HT receptors, i.e. 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors, which have been identified as being distributed in the spinal cord. Many studies have implicated 5-HT_{1A} and 5-HT_2 as being the most important in spinal antinociception mediated by the descending pain-inhibitory pathways [29]. Bardin et al. [3] showed that the antinociceptive effect of serotonin (1 μg, i.t.) was significantly attenuated by intrathecal pretreatment with ketanserin. In this report, the other subtypes of 5-HT receptors, such as 5-HT_{1A} and 5-HT_4 receptors, did not seem to be involved in the descending pain-inhibitory pathways, but 5-HT_2 receptors are known to play an important role. Sasaki et al. [36] showed that the

antinociceptive effects of DOI (a 5-HT_2 receptor agonist) were found to be completely antagonized by intrathecal pretreatment with ketanserin. Since intrathecally administered 5-HT produces antinociception in rats, rabbits, and cats as demonstrated by Yaksh and Wilson [54] in 1979, a number of subsequent studies highlighted the role of 5-HT_2 receptors in antinociception [14, 29, 36]. Although it is possible that 5-HT_{1A}, 5-HT_3, and 5-HT_4 receptors may also be involved in a minor proportion of the descending pain-inhibitory pathways, pretreatment with ketanserin to block 5-HT_2 receptors should be capable of shutting down most of the descending serotoninergic pathways, thus ruling out its impact on the potentiation of morphine antinociception by DM.

Although the binding of DM to opioid receptors was predicted not to occur at low concentrations, the possible allosteric effect of DM on opioid binding has never been formally investigated and reported. In the present study, we examined the possible effect of DM on μ-opioid receptor binding using [^{3}H]DAMGO as the radioactive ligand for the μ-opioid receptors, and found that the binding of morphine to μ-opioid receptors was not affected by DM. The concentration of DM applied in the competition binding assay was four times that of morphine, which was similar to the dosage ratio we used in vivo. The present results possibly rule out the direct interaction between DM and morphine at the opioid receptor level in correlation with the mechanism of potentiation of morphine antinociception by DM.

In summary, the present study shows that DM can potentiate morphine-induced antinociception at both the spinal and supraspinal levels. The mechanism underlying the potentiation of DM is possibly not related to the descending pain-inhibitory pathways, at least not to the adrenergic and serotoninergic pathways. The possible effect of DM on μ-opioid receptor binding can also be ruled out. In order to reveal the role of NMDA receptors in the effect of DM on potentiating morphine antinociception, we pretreated rats with MK-801 (5 or 10 μg, i.t.), and found that DM (2 μg, i.t.) was unable to further enhance the (0.5 μg, i.t.) antinociceptive effect of morphine (45.7 vs. 43.3–50.5%) [8]. This implies that spinal NMDA receptors may play an important role in the potentiating effect of DM on morphine antinociception. Although the detailed mechanism by which DM does this is still unknown, the present results revealed the clinical potential of DM as an adjunct drug with morphine, thereby reducing the dose of morphine required to achieve pain treatment from results of our previous clinical trial [6]. Because DM is a widely used and accepted antitussive with a completely safe profile, the clinical application of DM should be helpful in potentiating morphine analgesia and decreasing the dose of morphine to prevent adverse effects.

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References

1 Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT. The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. Science 234:734–737;1986.
2 Baker AK, Hoffmann VL, Meert TF. Dextromethorphan and ketamine potentiate the antinociceptive effects of mu- but not delta- or kappa-opioid agonists in a mouse model of acute pain. Pharmacol Biochem Behav 74:73–86; 2002.
3 Bardin L, Lavarenne J, Eschalier A. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. Pain 86:11–18;2000.
4 Bem JL, Peck R. Dextromethorphan. An overview of safety issues. Drug Saf 7:190–199; 1992.
5 Chevlen E. Morphine with dextromethorphan: conversion from other opioid analgesics. J Pain Symptom Manage 19:S42–49;2000.
6 Chia YY, Liu K, Chow LH, Lee TY. The preoperative administration of intravenous dextromethorphan reduces postoperative morphine consumption. Anesth Analg 89:748–752; 1999.
7 Chou YC, Liao JF, Chang WY, Lin MF, Chen CF. Binding of dimemorfan to sigma-1 receptor and its anticonvulsant and locomotor effects in mice, compared with dextromethorphan and dextrorphan. Brain Res 821:516–519;1999.
8 Chow LH, Huang EYK, Ho ST, Lee TY, Tao PL. Dextromethorphan potentiates morphine antinociception at the spinal level in rats. Can J Anaesth, in press.
9 Connor M, Christie MD. Opioid receptor signalling mechanisms. Clin Exp Pharmacol Physiol 26:493–499;1999.
10 D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79;1941.
11 Ebert B, Thorkildsen C, Andersen S, Christrup LL, Hjeds H. Opioid analgesics as noncompetitive N-methyl-D-aspartate (NMDA) antagonists. Biochem Pharmacol 56:553–559;1998.
12 Fairbanks CA, Stone LS, Kitto KF, Nguyen HO, Posthumus IJ, Wilcox GL. α(2C)-Adrenergic receptors mediate spinal analgesia and adrenergic-opioid synergy. J Pharmacol Exp Ther 300:282–290;2002.
13 Fardin V, Oliveras JL, Besson JM. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat. I. The production of behavioral side effects together with analgesia. Brain Res 306:105–123;1984.
14 Fone KC, Robinson AJ, Marsden CA. Characterization of the 5-HT receptor subtypes involved in the motor behaviours produced by intrathecal administration of 5-HT agonists in rats. Br J Pharmacol 103:1547–1555;1991.

15 Grass S, Hoffmann O, Xu XJ, Wiesenfeld-Hallin Z. N-Methyl-D-aspartate receptor antagonists potentiate morphine's antinociceptive effect in the rat. Acta Physiol Scand 158:269–273;1996.
16 Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 143:29–36;1982.
17 Hernandez SC, Bertolino M, Xiao Y, Pringle KE, Caruso FS, Kellar KJ. Dextromethorphan and its metabolite dextrorphan block $\alpha_3\beta_4$ neuronal nicotinic receptors. J Pharmacol Exp Ther 293:962–967;2000.
18 Hieble JP, Bondinell WE, Ruffolo RR Jr. Alpha- and beta-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. J Med Chem 38:3415–3444;1995.
19 Hoffmann O, Wiesenfeld-Hallin Z. Dextromethorphan potentiates morphine antinociception, but does not reverse tolerance in rats. Neuroreport 7:838–840;1996.
20 Horvath G, Joo G, Dobos I, Klimscha W, Toth G, Benedek G. The synergistic antinociceptive interactions of endomorphin-1 with dexmedetomidine and/or S(+)-ketamine in rats. Anesth Analg 93:1018–1024;2001.
21 Hough LB, Nalwalk JW, Leurs R, Menge WM, Timmerman H. Significance of GABAergic systems in the action of improgan, a non-opioid analgesic. Life Sci 68:2751–2757;2001.
22 Howe JR, Wang JY, Yaksh TL. Selective antagonism of the antinociceptive effect of intrathecally applied alpha adrenergic agonists by intrathecal prazosin and intrathecal yohimbine. J Pharmacol Exp Ther 224:552–558; 1983.
23 Kuraishi Y, Hirota N, Sato Y, Kaneko S, Satoh M, Takagi H. Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn. Brain Res 359:177–182;1985.
24 Larsen JJ, Arnt J. Spinal 5-HT or NA uptake inhibition potentiates supraspinal morphine antinociception in rats. Acta Pharmacol Toxicol (Copenh) 54:72–75;1984.
25 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275;1951.
26 MacPherson RD. The pharmacological basis of contemporary pain management. Pharmacol Ther 88:163–185;2000.
27 Martin TJ, DeMontis MG, Kim SA, Sizemore GM, Dworkin SI, Smith JE. Effects of beta-funaltrexamine on dose-effect curves for heroin self-administration in rats: comparison with alteration of [^{3}H]DAMGO binding to rat brain sections. Drug Alcohol Depend 52:135–147; 1998.
28 Murray TF, Leid ME. Interaction of dextrorotatory opioids with phencyclidine recognition sites in rat brain membranes. Life Sci 34:1899–1911;1984.
29 Obata H, Saito S, Sasaki M, Ishizaki K, Goto F. Antiallodynic effect of intrathecally administered 5-HT(2) agonists in rats with nerve ligation. Pain 90:173–179;2001.
30 Pang IH, Vasko MR. Effect of depletion of spinal cord norepinephrine on morphine-induced antinociception. Brain Res 371:171–176;1986.
31 Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. New York, Academic Press, 1986.
32 Plesan A, Hedman U, Xu XJ, Wiesenfeld-Hallin Z. Comparison of ketamine and dextromethorphan in potentiating the antinociceptive effect of morphine in rats. Anesth Analg 86: 825–829;1998.
33 Reddy SV, Yaksh TL. Spinal noradrenergic terminal system mediates antinociception. Brain Res 189:391–401;1980.
34 Redwine KE, Trujillo KA. Effects of NMDA receptor antagonists on acute mu-opioid analgesia in the rat. Pharmacol Biochem Behav 76: 361–372;2003.
35 Roychowdhury SM, Fields HL. Endogenous opioids acting at a medullary mu-opioid receptor contribute to the behavioral antinociception produced by GABA antagonism in the midbrain periaqueductal gray. Neuroscience 74:863–872;1996.
36 Sasaki M, Ishizaki K, Obata H, Goto F. Effects of 5-HT2 and 5-HT3 receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test. Eur J Pharmacol 424:45–52;2001.
37 Sawynok J. The 1988 Merck Frosst Award. The role of ascending and descending noradrenergic and serotonergic pathways in opioid and non-opioid antinociception as revealed by lesion studies. Can J Physiol Pharmacol 67:975–988;1989.
38 Steinberg GK, Bell TE, Yenari MA. Dose escalation safety and tolerance study of the N-methyl-D-aspartate antagonist dextromethorphan in neurosurgery patients. J Neurosurg 84:860–866;1996.
39 Tao PL, Lee HY, Chang LR, Loh HH. Decrease in mu-opioid receptor binding capacity in rat brain after chronic PL017 treatment. Brain Res 526:270–275;1990.
40 Tao PL, Lee CR, Law PY, Loh HH. The interaction of the mu-opioid receptor and G protein is altered after chronic morphine treatment in rats. Naunyn Schmiedebergs Arch Pharmacol 348:504–508;1993.
41 Tao PL, Liu CF, Tsai HC. Chronic intracerebroventricular administration of morphine down-regulates spinal adenosine A1 receptors in rats. Eur J Pharmacol 278:233–237;1995.
42 Tao PL, Seybold VS, Loh HH. Autoradiographic evidence for decrease in binding of mu- and delta-opioid receptors after subchronic [D-Ala2,D-Leu5]enkephalin treatment in rats. Eur J Pharmacol 231:145–149;1993.
43 Tao PL, Yeh GC, Su CH, Wu YH. Co-administration of dextromethorphan during pregnancy and throughout lactation significantly decreases the adverse effects associated with chronic morphine administration in rat offspring. Life Sci 69:2439–2450;2001.
44 Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251:85–87; 1991.
45 Tseng LL, Tang R. Differential actions of the blockade of spinal opioid, adrenergic and serotonergic receptors on the tail-flick inhibition induced by morphine microinjected into dorsal raphe and central gray in rats. Neuroscience 33: 93–100;1989.
46 Tsuchiya M, Yamazaki H, Hori Y. Enkephalinergic neurons express 5-HT3 receptors in the spinal cord dorsal horn: single cell RT-PCR analysis. Neuroreport 10:2749–2753;1999.
47 Wang JK. Antinociceptive effect of intrathecally administered serotonin. Anesthesiology 47: 269–271;1977.
48 Wang YY, Wu SX, Liu XY, Wang W, Li YQ. Effects of c-fos antisense oligodeoxynucleotide on 5-HT-induced upregulation of preprodynorphin, preproenkephalin, and glutamic acid decarboxylase mRNA expression in cultured rat spinal dorsal horn neurons. Biochem Biophys Res Commun 309:631–636;2003.
49 Weinbroum AA, Rudick V, Paret G, Ben-Abraham R. The role of dextromethorphan in pain control. Can J Anaesth 47:585–596;2000.
50 White PF, Way WL, Trevor AJ. Ketamine – its pharmacology and therapeutic uses. Anesthesiology 56:119–136;1982.
51 Wigdor S, Wilcox GL. Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. J Pharmacol Exp Ther 242:90–95;1987.
52 Wong CS, Hsu MM, Chou YY, Tao PL, Tung CS. Morphine tolerance increases [^{3}H]MK-801 binding affinity and constitutive neuronal nitric oxide synthase expression in rat spinal cord. Br J Anaesth 85:587–591;2000.
53 Wong CS, Liaw WJ, Tung CS, Su YF, Ho ST. Ketamine potentiates analgesic effect of morphine in postoperative epidural pain control. Reg Anesth 21:534–541;1996.
54 Yaksh TL, Wilson PR. Spinal serotonin terminal system mediates antinociception. J Pharmacol Exp Ther 208:446–453;1979.
55 Yang CY, Wong CS, Chang JY, Ho ST. Intrathecal ketamine reduces morphine requirements in patients with terminal cancer pain. Can J Anaesth 43:379–383;1996.