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Interaction of combined administration of intrathecal morphine with subcutaneous morphine or buprenorphine

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KEY WORDS morphine; buprenorphine; drug interaction

ABSTRACT

AIM: To analyze the mode of interaction of combined administration of intrathecal morphine with subcutaneous morphine or buprenorphine. **METHODS**: Different groups of rats were scheduled to undergo administration of intrathecal (ith) morphine, subcutaneous (sc) morphine, sc buprenorphine, and the combinations of ith morphine with sc morphine or buprenorphine in a series of dose ratios. Nociceptive responses of hind paws of each animal were measured by means of "plantar stimulation" test. The test latency was converted to the percent of maximal possible effect (% MPE). **RESULT**: Morphine ith, morphine sc, buprenorphine sc, as well as combinations in all dose ratios increased the % MPE in a dose-dependent manner. Isobolograms showed that the ED_{50} points determined for the combinations were plotted significantly left to the theoretical additive line. **CONCLUSION**: The combination of morphine ith with either morphine sc or buprenorphine sc resulted in a synergistic effect. This interaction might be due to the activation of the synergistic antinociceptive mechanisms between supraspinal and spinal levels.

INTRODUCTION

Opioids are potent analgesics which act upon wide regions of supraspinal central sites or spinal cord. However, the most dangerous side effect of respiratory depression limits the usage of opioids. If it is possible to obtain enhanced analgesic effect by the combination of opioids, it can be expected to minimize the notable side effects by decreasing the total doses. A previous study indicated that combined administration of morphine in the spinal cord and brain ventricles produced a supraadditive antinociception[1]. But clinical application of brain ventricular injection is almost impossible. It was also reported that supraadditive antinociception could be induced by simultaneous intrathecal and intraperitoneal morphine[2], and the effect of systemic opioids was mediated predominantly by supraspinal systems[3]. So, it might be expected that combined administration of spinal and systemic opioids may produce potentiated analgesic efficacy.

However, based on the pharmacological receptor theory, the interaction of drugs with varying degrees of intrinsic efficacy is dependent on their individual affinity to the receptors. When opioids with different intrinsic efficacy are combined, the effects of opioids with higher efficacy may be reduced by competitive occupation of the receptors. This hypothesis was proved by the experiment in which buprenorphine and morphine were injected intraperitoneally[4]. So, does the interaction between partial agonists and pure agonists of opioid receptors differ from that between pure agonists, in situations where these drugs are combined systemically and intrathecally?

To elucidate this, the present study was designed to analyze the mode of interaction of combined administration of intrathecal morphine with subcutaneous morphine or buprenorphine respectively.

MATERIALS AND METHODS

Animal preparation The experiments were performed using male, 300 - 350 g, Sprague-Dawley rats. Animals were housed individually in a temperature controlled room having a 12-h light-dark cycle, with both food and water available *ad libitum*. Tests were per-

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formed during the light cycle. Under halothane anesthesia, a chronic catheterization of the lumbar subarachnoid space was performed for the intrathecal injection. Briefly, a polyethylene 10 catheter was inserted through the atlanto-occipital membrane and pressed 10 cm caudal into the lumbar region of the spinal cord. The catheter was then fixed to the back of the neck of the animal. The day after the surgery, the animals who exhibited no signs of neurological deficit underwent the following experiment at procedure.

Nociceptive threshold test Nociceptive responses of hind paws of each animal were measured by means of "plantar stimulation" test using a device designed and built by Yaksh's laboratory[5] (University of California, San Diego, USA). The rats were placed in a clear plastic cage on an elevated floor of clear glass. A radiant heat source from a 50-W, 8-V lamp was contained in a movable holder placed beneath the glass floor. The radiant heat diameter was 4 mm and bulb intensity was controlled at 5.5 A. To initiate a test, the underfloor heat source was positioned to focus at the plantar surface of one hind paw which was completely in contact with the glass. The light was then activated meanwhile the timing circuit was initiated automatically. The nociceptive response was determined by the interval from the application of the light beam to the hind paw withdrawal. A cutoff time of three times of the baseline latency was set in order to avoid tissue injury.

Drugs protocol Each rat was used for one dose only. Initially, baseline nociceptive threshold was defined by the average of three measurements. Subsequently, drug administration was performed in a blind fashion. After drug injection, nociceptive response latency of the hind paw was measured at a 5-min interval until the baseline response was regained. The peak time of antinociceptive effect and the duration of the effect were determined. The whole protocol was divided into two parts: First, different groups of animals received either ith morphine, sc morphine or sc buprenorphine injection individually to conduct dose-response curves and to determine ED_{50} values of each drug. Secondly, other groups of animals underwent the combined administration of ith morphine with sc morphine or buprenorphine. The combinations were delivered according to the respective fractions of ED_{50} values (1/2, 1/4, 1/8 of ED_{50}), and the fractional dose combinations were conducted in a series of dose ratios such as 1:1, 1:2, and 2:1, for ith and sc injection. In this way, the dose-response curves and ED_{50} values of each combination were determined. All drugs were dissolved in Ringer's solution and administered in an injection volume of 1 $mL \cdot kg^{-1}$ for sc and 10 μL for ith. Following each ith drug injection, the catheter was refreshed by another 10 μL Ringer's solution to confirm the drugs entry into intrathecal space. For sc administration drugs were injected into the neck subcutis. In experiments using combined sc and ith opioids, injections was timed so that the peak effect of ith and sc administration would coincide. Three days after the experiment, each rat underwent the same dose injection to check the response. If the result was repeated, the data from the first experiment was used for statistical analysis.

Statistics The response latency was converted to the percent of maximal possible effect (% MPE) which was calculated by the formula:

$$\% \text{ MPE} = \frac{(\text{Postdrug response latency} - \text{Baseline response latency})}{(\text{Cutoff time} - \text{baseline response latency})}$$

Where postdrug response latency = the longest response latency observed after drug administration, baseline response latency = the average of three measures of the response latency before drug administration, and cutoff time = 15 s.

The dose-response curves of particular agents were obtained by plotting % MPE versus drug dose. ED_{50} values and 95 % confidence intervals were calculated by the linear regression. The comparison between groups was carried out with analysis of variance. A P value of < 0.05 was considered significant.

Isobolographic analysis was used to define the mode of the interaction between the drug classes according to the procedure of Tallarida[6]. It has the advantage of being independent of the slopes of the dose-response curves. The isobologram was constructed by plotting single-drug ED_{50} points on the dose coordinates of isobologram, and ED_{50} points of each combinations in the dose field. A straight line joining the single-drug ED_{50} points is termed the "additive line". If the ED_{50} of a combination falls on the theoretical additive line, the effect of the drug mixture is additive. The points to the left of the theoretical additive line would indicate a synergistic interaction. Whereas the points to the right of the line would indicate a subadditive or antagonistic interaction.

RESULTS

Time-response The time-response of the anti-

nociceptive effects produced by each agent at the typical dose is displayed in Fig 1. As indicated, the maximum effects produced by ith and sc injections were observed at 10 min and 20 min respectively after administration. Therefore, in experiments using combined ith and sc administration, ith injection was performed 10 min after sc injection in order to match the peak effects of individual drugs. The duration of the effect of each agent lasted for no longer than 60 min.

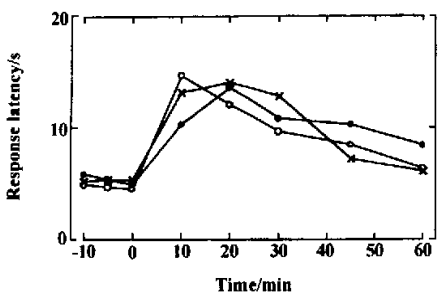

Fig 1. The time-response antinociceptive effects produced by each agent at the typical doses. The peak effect of ith morphine 10 μg (○), sc morphine 8 mg·kg^{-1} (●), or sc buprenorphine 0.5 mg·kg^{-1} (×), appeared at 10 min, 20 min, and 20 min after injection respectively.

Dose-response analysis Intrathecal morphine, sc morphine or sc buprenorphine resulted in a dose-dependent increase in the response latency. The ED_{50} values as well as 95 % confidence intervals of the individual drugs and all combinations are listed on Tab 1. Combined administration of ith morphine with sc morphine or buprenorphine resulted in a significant leftward and upward shift in the morphine dose-response curve. Furthermore, as the dose ratio of sc injection increased, more leftward and upward shifts in the morphine dose-response curve were observed (Fig 2).

Tab 1. ED_{50} values and 95 % confidence intervals of all drugs and combinations.

	ED_{50}	95 % *CI*
ith morphine (μg)	4.3	2.7-5.8
sc morphine (mg·kg^{-1})	5.1	3.9-6.2
sc buprenorphine (μg·kg^{-1})	104.0	54.9-153.1
ith mor + sc mor* (μg)		
1:1	1.4	1.0-1.8
1:2	0.9	0.7-1.1
2:1	1.9	1.3-2.4
ith mor + sc bupre* (μg)		
1:1	1.7	1.3-2.1
1:2	1.2	0.8-1.6
2:1	2.7	1.9-3.6

*ED_{50} values of the combinations were expressed as the ED_{50} of the ith morphine.

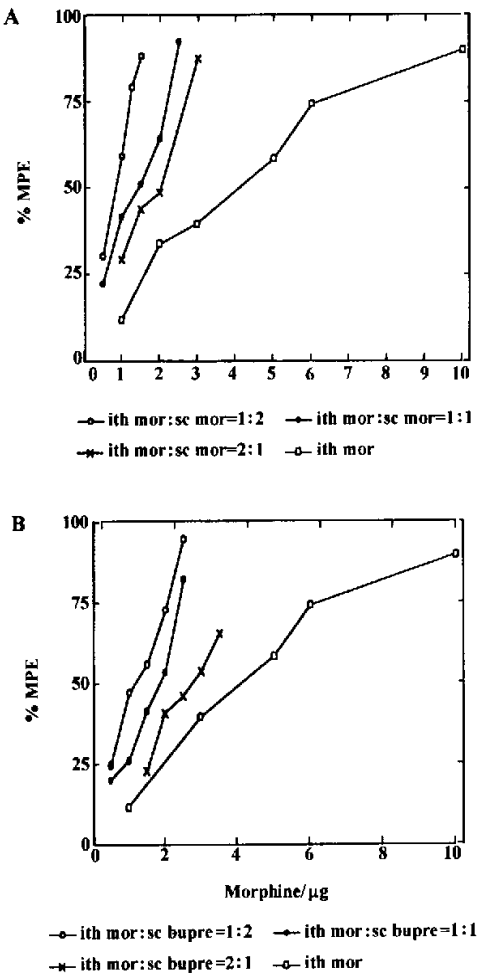

Fig 2. A) shows dose-response effects of combined administration of ith and sc morphine. B) shows dose-response effects of combined administration of ith morphine and sc buprenorphine. Both resulted in significant leftward and upward shift in the morphine dose-response curve. As the dose ratio of sc injection increased, more leftward and upward shifts were observed. ith:sc = 1:2 (○), ith:sc = 1:1 (●), ith:sc = 2:1 (×), ith alone (□).

Isobolographic analysis Morphine-morphine and morphine-buprenorphine isobolograms were construct-

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ed. The experimental combination ED_{50} points determined for morphine-morphine combination in all three dose ratios were found to be plotted significantly left to the theoretical additive line indicating synergistic interaction between ith and sc morphine. For morphine-buprenorphine coadministration, the experimental combination ED_{50} points for the dose ratio of 1 : 1 and 1 : 2 (ith : sc) were significantly left to the theoretical additive line, the ED_{50} points for the mixture in the dose ratio of 2 : 1 were on the left but not significantly, indicating the synergistic interaction presented when the sc buprenorphine dose was higher.

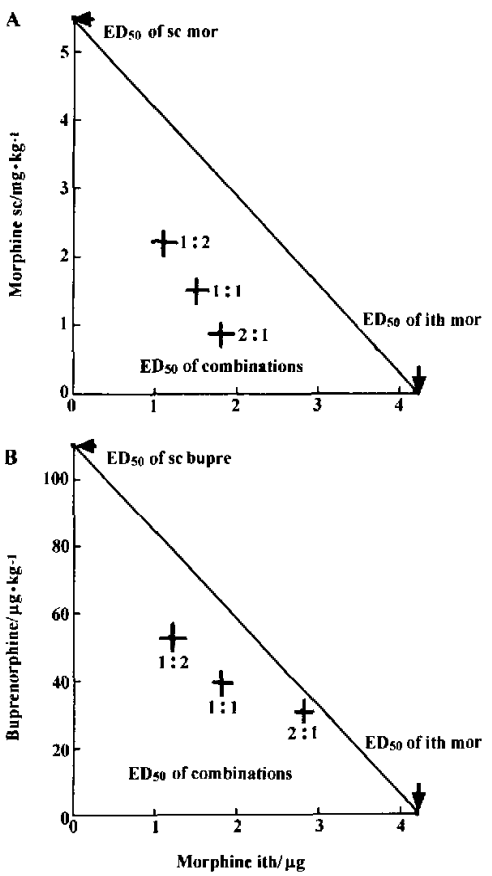

Fig 3. Isobologram of ED_{50} for various dose ratios of combination of ith morphine and sc morphine (A), or ith morphine and sc buprenorphine (B).

DISCUSSION

The drugs injected into rat's subcutis are estimated to be almost completely absorbed into the blood circuit and the effect of absorbed opioid has been proved to be mediated predominantly by supraspinal systems[3]. Previous experiments have shown that ith injection of morphine administered in the same volume as used in this study could not result in detectable diffusion of the drug to supraspinal structures[7]. So the antinociceptive effect induced by the combined injections in this study could be considered as the combined effect mediated from supraspinal and spinal system.

It has been demonstrated that an antinociceptive circuit exists between supraspinal and spinal levels[8]. This circuit has been called the central descending antinociceptive control mechanism[9]. When morphine concomitantly elicits anlagesia in multiple regions, its actions are dramatically potentiated[10]. The present study using dose-response and isobolographic analysis demonstrated that co-administration of inthrathecal and subcutaneous pure agonist of μ-opioid receptor (morphine) resulted in synergistic antinociceptive effect which was dependent on the dose ratio of sc injection. This finding is in accordance with the above mentioned reports.

Buprenorphine is a selective partial agonist of μ-opioid receptor and it's pharmacological effect is mediated predominantly by μ_1-receptor[11]. Buprenorphine has extremely high affinity but limited efficacy on μ-receptor. When given alone, its effects are similar to those of morphine. But when given together with morphine, it competes with the pure agonist and causes antagonism against the effect of morphine. However, the result of the present study could not be explained by this receptor theory. Dose-response and isobolographic analysis indicated a synergistic effect produced by co-administration of ith morphine and sc buprenorphine, which increased with an increase in the sc dose. We postulated that the combined effect might be induced by the synergism existing between supraspinal and spinal opioid sensitive sites. When the central descending antinociceptive control mechanism gets activated by systemically administered buprenorphine, the synergism between supraspinal and spinal levels overtake the competitive antagonism between partial and pure agonist. Therefore, the supraadditive effect appears. When the systemic dose is increased, the supraspinal regulatory effect becomes more predominant, and the synergism was more potent.

The respiratory depression induced by opioids is mediated by the same receptor as that of antinociception. Unfortunately, no clinically available opioid agent acts only on the respective subtypes of receptors which are special for respiratory depression or analgesia. These

two sites show equiefficacy to opiods. Partial agonists show lower incidence of respiratory depression. From this point of view, the most important contribution of the findings from present study was to demonstrate a clinically available route of combined administration of opioids to produce potent pain relief and to minimize the incidence of respiratory depression by decreasing the effective doses. In addition, using buprenorphine instead of morphine systemically may offer advantage of providing more reduction in respiratory depression than morphine with no danger of reducing antinociceptive effect.

In conclusion, the current study demonstrated that co-administration of intrathecal morphine combined with subcutaneous morphine or buprenorphine resulted in a synergistic antinociceptive effect, which was mediated predominantly by subcutaneous route. This interaction might due to the concurrent activation of spinal as well as supraspinal antinociceptive mechanisms.

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吗啡与丁丙诺啡的蛛网膜下腔与皮下联合给药的相互作用

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关键词 吗啡; 丁丙诺啡; 药物相互作用

目的: 观察大鼠吗啡与丁丙诺啡(或吗啡)的蛛网膜下腔与皮下联合给药的相互作用. **方法**: SD大鼠, 置入蛛网膜下腔导管. 辐射热诱发鼠腿撤退试验测痛阈. 分别蛛网膜下腔给予吗啡、皮下注射丁丙诺啡(或吗啡)、蛛网膜下腔给予吗啡与皮下注射丁丙诺啡(或吗啡)的联合给药. **结果**: 单独和联合给药均剂量依赖性地提高鼠痛阈. 联合给药的量效曲线的斜率均显著大于吗啡单独给药的曲线斜率. 等效线图显示联合给药的 ED_{50} 均位于理论推测的叠加效应线的左侧. **结论**: 吗啡与丁丙诺啡(或吗啡)的蛛网膜下腔与皮下联合给药呈协同效应.

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