学 位 論 文 の 要 旨

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学 位 論 文 名　Enhancement of Morphine Analgesic Effect with Induction of μ-Opioid Receptor Endocytosis in Rats

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論 文 内 容 の 要 旨

INTRODUCTION

Although morphine is a highly effective analgesic that has been used for centuries, its therapeutic use is often limited by severe side effects such as respiratory depression, tolerance, and dependence. Enormous effort has been invested to find a means of enhancing its analgesic efficacy and reducing undesirable side effects of morphine.

Analgesic effects of morphine are mediated by the μ-opioid receptor (MOR). Exposure of MOR to their agonists often results in rapid attenuation of receptor responsiveness. The process, acute desensitization, impairs signaling efficiency. It is usually followed by internalization of the receptor to resensitize or rapidly recover the cell responsiveness to agonists. However, morphine is unique in that it fails to cause internalization of the receptor after binding. Responsiveness to morphine can be enhanced by induction of MOR endocytosis if the unusual property limits acute analgesic effects of this opiate drug by desensitizing MOR while neither internalizing nor resensitizing the receptor. Recently, a low dose of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO), a specific MOR agonist, was reported to be able to evoke morphine-induced MOR internalization in rat spinal dorsal horn. In this study, we hypothesized that morphine-induced

analgesic effect can be enhanced by induction of MOR internalization and tested the hypothesis using coadministration of morphine with small doses of endocytosis-inducing agonists to facilitate morphine-mediated MOR internalization.

MATERIALS AND METHODS

Nociceptive test. Rats were administered with analgesics from implanted intrathecal catheters. The hot plate test was performed to measure the response to heat stimuli (52.0 ℃) by monitoring latency until hindpaw licking. The latency was converted to the percentage of the maximal possible effect (%MPE), calculated as (postdrug value – baseline value) / (cut-off value – baseline value) × 100%.

Quantification of MOR internalization. After the test, the rat was killed quickly and the spinal cord was dissected out. The tissue was sectioned and stained with anti-MOR and anti-NeuN antibodies. MOR distribution in dorsal horn neurons was examined with a confocal laser scanning microscope. At least 100 neurons were counted per rat.

RAVE value. A concept RAVE is defined as the relative activity versus the ability of a ligand to induce endocytosis *in vitro*. We determined the RAVE value *in vivo*; the activity and endocytosis were expressed as %MPE and %internalized neurons, respectively.

RAVE value = %MPE / %internalized neurons

Statistical analysis. Data are presented as mean ± SEM. Statistical analyses were one-way ANOVA followed by Scheffe post-test. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

We first characterized fentanyl-induced internalization of MOR in the spinal dorsal horn *in vivo*. In fentanyl-treated rats, MOR immunoreactivity was depleted from the plasma membrane and observed in numerous endosomes within the dorsal horn neurons. We showed for the first

time that intrathecal fentanyl produced MOR internalization in rat spinal dorsal horn neurons together with analgesic effect in a dose dependent manner. The order of the RAVE values was DAMGO < fentanyl < morphine *in vivo*.

DAMGO (5 ng) or fentanyl (0.5 μg) produced neither the detectable MOR internalization in the dorsal horn nor significant antinociception. The combination of the small amounts of DAMGO and fentanyl with a submaximal antinociceptive dose of morphine (2.5 μg) increased MOR internalization in the dorsal horn to 62.8% and 33.3%, respectively. Moreover, under the same conditions, the antinociceptive effect of the combination of DAMGO and fentanyl with morphine was greatly enhanced to 94.0% and 70.0%, respectively. Based on the hypothesis that potentiation of morphine-induced endocytosis can enhance its antinociceptive effect, MOR agonists with higher efficacy to induce MOR internalization, or with lower RAVE value, would be favorable to improve morphine analgesia. Actually, our results showed that DAMGO, which has a lower RAVE value than fentanyl, enhanced the analgesic effect of morphine more strongly than fentanyl. The enhancement of the analgesic effect by the combination of morphine with other opioids could reduce the amount of morphine to obtain satisfactory antinociception, and thus may prevent the development of tolerance to morphine. Also, the combination-induced enhancement of analgesic effect may be one of the mechanisms of the restored opioid responses observed on opioid switching.

CONCLUSION

This study demonstrated that coadministration of a low dose of MOR-internalizing agonist resulted in the potentiation of morphine-induced endocytosis and analgesia, and suggests that the coadministration of morphine with MOR-internalizing agonist is clinically applicable to develop successful pain management regimens to achieve satisfactory analgesia using less morphine.