Rapid Communication

Analgesic action of loperamide, an opioid agonist, and its blocking action on voltage-dependent Ca\(^{2+}\) channels

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Abstract

We investigated the relationship between the antinociceptive effect of the opiate agonist loperamide at the spinal level and its inhibitory effect on calcium influx. Intrathecal administration of loperamide showed a significant antinociceptive effect in the formalin test, which was not prevented by naloxone. On the other hand, no significant effects were observed by nicardipine, an L-type specific blocker, or by BAY K8644, an L-type specific agonist, suggesting no significant role of L-type calcium channels in nociceptive signal transduction. Loperamide suppressed the calcium influx in dorsal root ganglion neurons. As the antinociceptive effect of loperamide was not affected by naloxone or other calcium channel blocking toxins, and loperamide showed a direct inhibitory effect on calcium-influx, the analgesic effect of intrathecally injected loperamide might be due to its blockade of the voltage-dependent calcium channels at the terminals of the primary afferent fibers.

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1. Introduction

Loperamide is a widely used antidiarrheal agent (Niemegeers, et al., 1974) that has high affinity for µ-opioid receptors (DeHaven-Hudkins et al., 1999), but does not have analgesic effects when administered systemically (Niemegeers, et al., 1979) because it penetrates into the central nervous system poorly (Wuster and Herz, 1978). It also inhibits voltage-dependent calcium channels. It blocks Ca\(^{2+}\) currents in cultured hippocampal pyramidal neurons of mice (Church et al., 1994). Although intrathecal injection of loperamide has analgesic effect, the exact relationship between its analgesic action and its inhibitory effect on voltage-dependent calcium channels at the dorsal horn, where the primary afferent fibers project, is still unclear.

The aim of this study was to clarify the antinociceptive action of intrathecal injection of loperamide in mice, and to examine the relationship between its analgesic action and its inhibitory effects on voltage-dependent Ca\(^{2+}\) channels at the primary afferent fibers.

2. Materials and methods

2.1. Formalin test

The subjects were 4–6-week-old mice, maintained on an alternating 12-h light:12-h dark cycle. Before the formalin test, unanesthetized mice were given intrathecal injections using the technique of Hylden and Wilcox (1980); the formalin test consisted of injecting 20 μl of 2.0% formaldehyde solution subcutaneously (Murakami...
et al., 2000). The behavioral experiments were performed with the approval of the Ethics Committee for Animal Experiments of Tohoku Pharmaceutical University. Significant differences from controls were determined by Student’s $t$-test. Dunnett’s multiple comparison was used for the multiple intergroup tests.

2.2. Intracellular calcium measurement

Dorsal root ganglion (DRG) cells were isolated from newborn mice (Murakami et al., 2000). The increase in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) in single cells was measured by the fura-2 method (Gryniewicz et al., 1985) with an ARGUS-50 system (Hamamatsu Photonics, Hamamatsu, Japan) with band pass filters of 340 and 380 nm, using a balanced salt solution with the following composition (mM): NaCl 150, KCl 5, CaCl$_2$ 1.2, MgCl$_2$ 1.2, d-glucose 10, and HEPES 25 (pH 7.4). Naloxone pretreatment (5 μM) was done 10 min prior to the application of loperamide.

3. Results

Pretreatment with intrathecal loperamide 10 min before administering subcutaneous formalin significantly reduced the licking time in the early and late phases (Fig. 1). This analgesic effect was comparable with that of other calcium channel blockers, such as ω-conotoxin GVIA and ω-agatoxin IVA, which specifically block N- or P/Q-type calcium channels, respectively. Conversely, nicardipine, a specific blocker of L-type channels, showed no effect (Fig. 1A). Furthermore, BAY K8644, which is an L-type calcium channel agonist, revealed no significant effect on licking behavior in formalin test.

Loperamide showed dose-dependent effect in both early and late phases (Fig. 1B). To further investigate the mechanism of its analgesic effect, naloxone, which is an opioid receptor antagonist, was intraperitoneally administered. Naloxone (10 mg/kg) had no significant effect on the analgesic effect of loperamide, which suggest that the analgesic effect of loperamide in the

Fig. 1. (A) Comparative study of the analgesic effect of loperamide with calcium channel blockers (ω-ATXIVA: ω-agatoxin IVA 0.2 nmol/kg; ω-CTXGVIA: ω-conotoxin GVIA 0.5 nmol/kg; LOP: loperamide 300 nmol/kg; NIC: nicardipine 300 nmol/kg; BAY: BAY K8644 300 nmol/kg). (B) The effect of intrathecal loperamide (0.3, 3, 30, and 300 nmol/kg). Nociceptive behavior in both the early (0–10 min; upper panel) and late (10–30 min; lower panel) phases is shown as the amount of time spent licking the injected paw.
The present study is independent of its effect on opioid receptors (Fig. 2A). In addition, we examined combined effects of loperamide and calcium channel blocking toxins ($\nu$-conotoxin GVIA or $\nu$-agatoxin IVA). No clear additive effects were observed, suggesting that both toxins might share the same pharmacological pathway with loperamide (Fig. 2B).

To examine the relationship between loperamide’s analgesic effect and its inhibitory effect on calcium channels, we further examined the inhibitory effect of loperamide on Ca\(^{2+}\) influx in response to 90 mM KCl. Membrane depolarization with 90 mM KCl increased [Ca\(^{2+}\)], in DRG neurons, which are known to have endogenous L-, P/Q-, and N-type calcium channels, as shown in Fig. 3A (Fujii et al., 1997). Loperamide dose-dependently inhibited this [Ca\(^{2+}\)] increase. At the highest dose (10 $\mu$M), which is comparable to the dosage of dihydropyridine derivatives (Murakami et al., 2000), loperamide produced 95.0% inhibition (Fig. 3B), while other calcium channel blockers, such as nicardipine, $\omega$-conotoxin GVIA and $\omega$-agatoxin IVA, also showed inhibitory effects (Fig. 3Bii). A saturating concentration of nicardipine (1.0 $\mu$M) to block L-type calcium channels revealed a significant reduction (48.4%) in response to high KCl stimulation. One $\mu$M $\omega$-conotoxin GVIA, a dosage that should completely block N-type currents, produced a 33.2% reduction in response to high KCl stimulation, suggesting that 33.2% of the Ca\(^{2+}\) influx was due to N-type currents. A saturating amount of $\omega$-agatoxin IVA (0.1 $\mu$M) reduced the [Ca\(^{2+}\)] increase by 46.7%. Since its inhibitory effect was so profound, our results strongly suggest that loperamide blocks at least these three types of calcium channels in DRG neurons. Furthermore, to analyze the involvement of the opioid receptors in the inhibitory effect of loperamide on the Ca\(^{2+}\) influx, we next analyzed the effect of naloxone. Naloxone tended to decrease the inhibitory effect of loperamide, although the effect was not statistically significant (Fig. 3Biii), suggesting at this dosage, the inhibitory effect on the Ca\(^{2+}\) influx of loperamide is mainly due to its direct effect on the voltage-dependent calcium-channels.

4. Discussion

In previous studies, we showed that N- and P/Q-type calcium channels are highly expressed in murine primary...
afferent neurons. Some dihydropyridine analogues, such as amlodipine and cilnidipine, block the N-type current, and have an analgesic effect in the formalin test (Murakami et al., 2000, 2001). Furthermore, autoradiographic studies have shown that N-type channels are highly localized in superficial laminae I and II of the spinal cord, where the small myelinated (laminae I) and unmyelinated (laminae II) nociceptive afferents terminate (Gohil et al., 1994; Kerr et al., 1988). Therefore, N- and P/Q-type Ca\(^{2+}\) channels appear to be involved in signal transduction in nociceptive afferent fibers. Considering the strong effect of loperamide on the Ca\(^{2+}\)
influx that we observed in this study and the non-inhibitory effect by naloxone, at least a part of the antinociceptive action of the intrathecally delivered loperamide might be related to its inhibitory effect on Ca\(^{2+}\) influx at the terminals of nociceptive primary afferents. On the other hand, as no significant antinociceptive effects were observed by nicipidine or BAY K8644, L-type calcium channels probably play no significant roles in nociceptive signal transduction at the spinal level.

In the present study, we found that intrathecally injected loperamide had antinociceptive effect in mice, which might be related to its inhibitory effect on calcium-influx. On the other hand, loperamide is known to have analgesic effect by its peripheral injection, which is dependent on naloxone (Shannon and Lutz, 2002). The reason of these discrepant results might be related to administration routes, but further analysis focused on this point is necessary to clarify the antinociceptive mechanism of loperamide and to find the most effective route for its future clinical application as analgesia. As there are no voltage-dependent calcium channels at the peripheral terminals of the primary afferent fibers but abundant numbers of calcium channels exist in the dorsal horn, where proximal terminals of afferent fibers project, we think intrathecal injection of loperamide tends to block calcium channels in the dorsal horn more effectively than peripheral injection, whose antinociceptive effect is probably due to its action at peripheral opioid receptors.

Formalin produces nociceptive stimuli and provides a model of chemical stimulation. The response to formalin stimulation is biphasic: the early phase corresponds to the response to the direct stimulation of the nerve endings, and the late phase results from the subsequent inflammation (Dubuisson and Dennis, 1977). Loperamide showed significant effects in both the early and late phases, suggesting that it acts on both mechanisms of nociception.

In summary, intrathecal administration of the opiate agonist loperamide had an antinociceptive effect, which might be independent of opioid pathway, with a profound inhibitory effect on Ca\(^{2+}\) influx in DRG neurons.

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