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研究テーマ 炎症性疼痛刺激による脊髄遺伝子由来のFOS蛋白発現に対するくも膜下Endomorphin投与の影響

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Isobolographic analysis of interaction between spinal endomorphin-1, a newly isolated endogenous opioid peptide, and lidocaine in the rat formalin test

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Abstract

Endomorphin-1, a newly isolated endogenous opioid ligand, has a potential affinity with mu-opioid receptor. We investigated antinociception of intrathecal endomorphin-1 and lidocaine in the rat formalin test and examined the interaction between the two agents using isobolographic analysis. Intrathecal endomorphin-1 caused dose-dependent suppression of the formalin-induced biphasic behavioral response. Intrathecal lidocaine produced dose-dependent inhibition of phase-2 behavioral response. Isobolographic analysis confirmed that combination of intrathecal endomorphin-1 and lidocaine, given at a fixed dose ratio, produced synergistic suppression of phase-2 behavioral response. These data demonstrate that spinal endomorphin-1synergistically interacts with local anesthetic lidocaine in producing antinociception in the formalin test.

Keywords: Endomorphin-1; Lidocaine; Formalin test; Isobolographic analysis

Subcutaneous injection of dilute formalin into the rat hind-paw produces a biphasic nociceptive response; phase 1 reflects an acute pain response and phase 2 is responsible to the injury-induced sensitization and hyperalgesia [3]. Several classes of agents act spinally to alter nociceptive processing. Lidocaine and mu-opioid receptor agonists produce a powerful antinociception by an inhibition of nociceptive C-fiber activity [6]. Spinal morphine and lidocaine have been shown to produce a depression of the behavioral response in the formalin test [9,20]. The aim of the combination of two drugs is to produce synergistic antinociceptive effects and to reduce the amount of each drug and thereby minimize the incidence and severity of side effects. Basic study showed antinociceptive interactions between intrathecal opioid agents and local anesthetics in rats using hot plate model [11]. To our knowledge, no study of interaction between intrathecal opioid agents and lidocaine in the formalin test has been conducted. Endomorphin-1, a newly isolated endogenous opioid ligand, has a potential affinity with mu-opioid receptor. In this study, we sought to: (1) define the effects of intrathecal endomorphin-1 and lidocaine on behavioral response of formalin test and (2) characterize the spinal interaction between the two agents using isobolographic analysis.

The following studies were carried out under a protocol approved by the Animal Experiment Committee of our College. Chronic intrathecal catheters were implanted in male Sprague–Dawley rats (250–350 g) under the isoflurane anesthesia. Briefly, through an incision in the atlanto-occipital membrane, a polyethylene (PE-10) catheter, filled with 0.9% saline, was advanced 8.5cm caudally to position its tip at the level of the lumbar enlargement. The rostral tip of the catheter was passed subcutaneously, externalized on top of the skull, and sealed with a stainless steel plug. Animals showing neurological deficits after implantation were excluded.

For formalin injection, 50 μl of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw using 27-G needle. Animals were then placed in a clear plexiglas cylinder (20 × 30 cm) for observation. A mirror was placed below the floor (plexiglas) at a 45° angle for unencumbered observation during the test. Pain-related behavior was quantified by counting the number of flinches for 1-min periods at 1–2 and 5–6 min (phase 1), and then at 5-min intervals during the period from 10 to 60 min (phase 2) after the formalin injection. Criteria for exclusion from

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the study included incomplete formalin injection, or excessive bleeding from injection site.

Drugs used in the study included endomorphin-1 (Tocris, UK) and lidocaine hydrochloride (RBI). As determined in preliminary studies, endomorphin-1 and lidocaine were administered intrathecally 20 and 5 min prior to formalin test, respectively, so that the peak effect of each drug coincided. The agents were delivered with a microsyringe in a total volume of 10 μl followed immediately by a 10 μl saline to flush the catheter. All agents were dissolved in saline.

In the formalin test, time-response data are presented as the mean ± SEM per minute. For the dose response analysis, data from phase 1 and phase 2 were considered separately. The effective dose producing a 50% reduction of flinching response of control was defined as the inhibitory dose 50 (ID_{50}). The log dose response lines were fitted using least square linear regression, the ID_{50} and 95% confidence interval (CI_{95}) for each drug being calculated.

Isobolographic analysis for drug-drug interaction was conducted according to the procedure of Tallarida et al. [18]. To perform the isobolographic analysis, endomorphin-1 and lidocaine were administered in combination as fixed ratios of the ID_{50} dose for each drug (1 nmol: 20 μg of endomorphin-1: lidocaine). The experimental ID_{50} value and CI_{95} for drug combination were calculated. The isobolos were drawn by plotting the experimental determined ID_{50} value of lidocaine on the *x*-axis and that of endomorphin-1 on the *y*-axis, delivered alone and in combination. The theoretical additive ID_{50} dose was calculated according to Tallarida [17]. For statistical comparison of the difference between the experimentally derived ID_{50} value and the theoretical additive value, Student's *t*-test was used. To describe the magnitude of the interaction, a total dose fraction value was calculated according to Malmberg and Yaksh [10].

Intrathecal endomorphin-1 at the doses used in the study did not affect motor function during the observation period (60 min). Intrathecal lidocaine dose-dependently resulted in a motor dysfunction. The motor dysfunction was reliably localized and forelimb function was unaffected. Fifteen minutes after injection of lidocaine, motor function recovered to normal. Thus, considering that formalin was injected at 5 min after administration of intrathecal lidocaine and that phase 2 begins at 10 min after injection of formalin, we think that the motor dysfunction is not sufficient to affect observation of phase 2 response of formalin test.

Fig. 1 showed that the time course of endomorphin-1 and lidocaine on the formalin test. Fig. 2 showed that endomorphin-1 and lidocaine alone produced a dose-dependent suppression of the behavioral response induced by formalin. ID_{50} (CI_{95}) values of endomorphin-1 in phase 1 and 2 were 12.5 (7.5–19.8) nmol and 18.6 (10.2–30) nmol, respectively. ID_{50} value of lidocaine in phase 1 was not calculated because rats showed motor dysfunction during the phase1. ID_{50} (CI_{95}) values of lidocaine in phase 2 was 365 (245–540) μg. The isobologram of combination of endomorphin-1 and

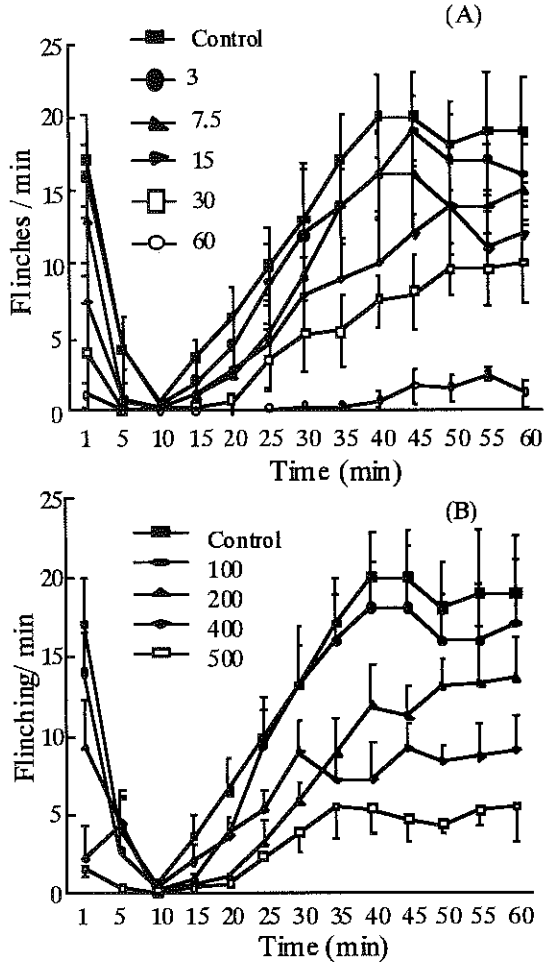

Fig. 1. Time-effect curve of intrathecal endomorphin-1 (nmol) and lidocaine (μg) administered before formalin. (A) Endomorphin-1 and (B) lidocaine. The number of flinches per minute is plotted vs. the time after the formalin injection into the hindpaw. Each line on the graph represents the mean ± SEM from eight to 12 rats.

lidocaine showed that the experimentally derived ID_{50} value decreased below the theoretical dose-additive line, and CIs of the theoretical additive point and those of the experimental point did not overlap (Fig. 3). This result indicated a significant difference between the experimental ID_{50} point and the theoretical additive ID_{50} point ($P < 0.05$) and a synergistic interaction between endomorphin-1 and lidocaine in the rat formalin test. The total dose fraction value in phase 2 was 0.28, which was less than 1, indicating a synergistic interaction. Even when the endomorphin-1 was given such that the time of peak pharmacological effect overlapped with the time of peak lidocaine effect, there was no enhancement in motor dysfunction.

This study clearly has shown the following: (1) intrathecal endomorphin-1 and lidocaine cause dose-dependent suppression of the behavioral response in the rat formalin test; and (2) at doses that do not affect motor function, the combination of endomorphin-1 and lidocaine produces synergistic antinociceptive interaction.

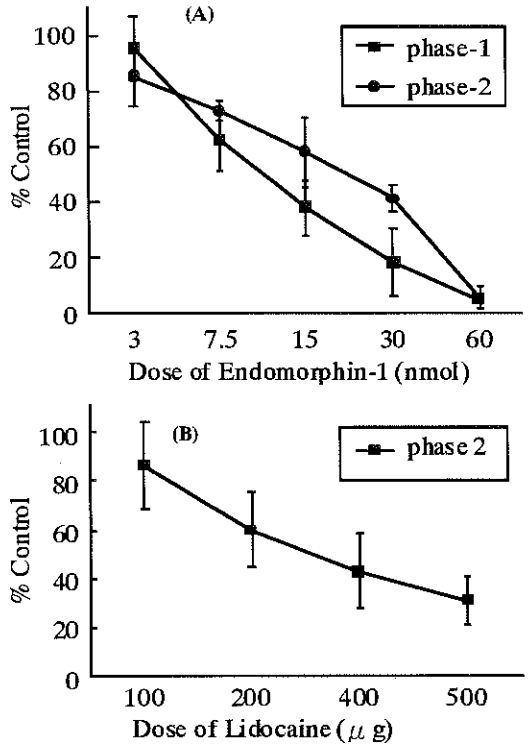

Fig. 2. Dose-response curves for intrathecal endomorphin-1 (A) and lidocaine (B). Mean values for biphasic activities expressed as a percent of control for endomorphin-1 and lidocaine.

The first phase of formalin test is representative of an acute effect mediated by the activation of nociceptive afferent C-fiber; the second phase is a composite of the ongoing barrage plus the generation of a facilitated state thought to result from the sensitization of the spinal cord (wind-up) [4]. Wind-up phenomenon is mediated partly by glutamate receptor of *N*-methyl-D-aspartate (NMDA) type [2].

Electrophysiologically, Dickenson and Sullivan [5] observed that injection of formalin resulted in a profound augmentation in the discharge of WDR neurons in rats and that the spinal administration of selective mu opioid receptor agonist before formalin injection blocked the augmentation. But this inhibition is obtained only when the agonist is given at doses that block the early C-evoked component [5]. A recent study showed that intrathecal endomorphin-1 inhibited the C-fiber activity in a dose-dependent manner [1]. There is direct evidence indicating that lidocaine selectively reduces the neuron activity evoked by C-fiber in rat spinal cord through decreasing NMDA receptor-mediated post-synaptic depolarization [13]. Importantly, electrophysiologic evidence showed that in combination with a low dose of opioid, lidocaine produced a highly marked potentiation of the inhibitions of the C-fiber evoked responses compared to either agent alone [6].

Behaviorally, a study showed that spinal endomorphin-1 produced a suppression of biphasic responses in the rat formalin test, but the effect was not dose-dependent [15]. However, the current study shows that the effect of intrathecal endomorphin-1 is readily does-dependent, which is consistent with electrophysiological study [1]. Intrathecal lidocaine produced suppression of behavioral response in the formalin test [3,9]. Although the interaction of morphine and lidocaine showed supra-additive effect in the hot plate test, the current study demonstrates that the interaction between endomorphin-1 and lidocaine is synergistic in nature by isobolographic analysis in the rat formalin test.

Synergistic interaction can occur when drugs affect different critical points along a common pathway. Although the principle effect of lidocaine remains on voltage-sensitive sodium channels, it may interact with voltage-sensitive K^+ and Ca^{2+} channels [8,14]. Binding studies have emphasized that opioid receptors are located presynaptically on the these small afferent terminals and these receptors mediate the inhibition of release of C-fiber peptide neurotransmitters (such as substance P and calcitonin gene related peptide) by the blockade of the activation of voltage sensitive Ca^{2+} channels [16]. A recent study demonstrated that endomorphin-1 induced Ca^{2+} channel inhibition by selectively activating the mu-opioid receptor [12]. Endomorphin-1 produced membrane hyperpolarization and suppression of excitatory postsynaptic potential on dorsal horn neuron [19] and also activated an inward potassium current [7]. Although the mechanisms of synergism between lidocaine

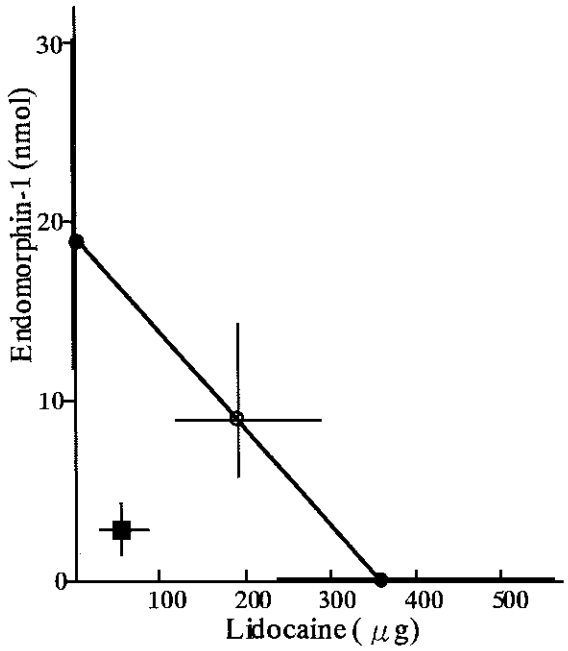

Fig. 3. Isobologram showing the interaction between intrathecal endomorphin-1 and lidocaine on phase 2 of formalin test. The ID_{50} values of lidocaine and endomorphin-1 are plotted on the *x*- and *y*-axis, respectively. The line connecting the ID_{50} points is the theoretical additive line, and the theoretical additive point (○) for the drug combination is shown on the additive line. The experimental ID_{50} value (■) of combination of the two agents was significantly lower than the theoretical additive value ($P < 0.05$), and CI_{95} did not overlap, indicating a synergistic interaction.

and endomorphin-1 remain unknown, it is likely that effects on sodium, calcium, potassium channels and neurons membrane hyperpolarization, play contributory roles.

In conclusion, the current study characterizes that intrathecal endomorphin-1 and lidocaine produce antinociceptive effects in a dose-dependent fashion in the formalin test and that the antinociceptive, synergistic interaction is observed between endomorphin-1 and lidocaine by isobolographic analysis. The clinical implications of this study are important in defending the use of intrathecal drug combination for improved pain management.

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