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Effects of a combination of ketanserin and propranolol on inflammatory hyperalgesia in rats

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ABSTRACT

Pain management is still challenging in clinic as current analgesics either are not very effective or produce serious adverse effects. This study aimed to examine if old drugs could display the new use and to develop a novel therapy for inflammatory pain. Injection of carrageenan in hindpaw evoked hyperalgesia detected by noxious heat stimulation. Intraplantar (i.pl.) injection of the 5-HT_{1A} receptor antagonist WAY-100635 increased paw withdrawal latency (PWL) above normal level (hypoalgesia) during the late phase of carrageenan-evoked inflammation. The hypoalgesia was completely abolished by systemic injection of naloxone chloride and naloxone methiodide. Moreover, i.pl. injection of a combination of WAY-100635 and ketanserin, a 5-HT_{2A} receptor antagonist, at their minimal doses attenuated hyperalgesia in the late phase of carrageenan-evoked inflammation. Subcutaneous (s.c.) injection of both ketanserin and propranolol dose-dependently inhibited carrageenan-evoked hyperalgesia. The treatment with a combination of ketanserin and propranolol by s.c. injection abolished carrageenan-evoked hyperalgesia at the doses, at which the drugs failed to alter the hypersensitivity when they were given alone. Furthermore, the combination of ketanserin and propranolol was also effective in relieving arthritic hyperalgesia and muscle pain at a minimal dose. The present study suggests that the activation of 5-HT_{1A} receptors suppressed naloxone-reversible antinociception contributing to the maintenance of inflammatory pain, and that the concomitant blockade of 5-HT_{1A} and 5-HT_{2A} receptors in the periphery produced synergistic effects on inflammatory hyperalgesia. It is proposed that the combination of ketanserin and propranolol injected s.c. could be a promising therapy for relieving inflammatory pain with minimal side effects.

1. Introduction

Pain is the most common symptom that pushes patients to seek medical help. Although pain is a protective mechanism warning that the body needs cares, chronic pain loses protective significance and profoundly affects life quality of patients. Available analgesics either are not very effective or produce serious adverse effects. For example, the utility of NSAID is limited to the management of mild-to-moderate pain (Katz, 2002). The family of NSAID is plagued by a number of lethal and non-lethal adverse effects, such as serious gastrointestinal bleeding and protein loss (Zeino et al., 2010). Opioids are the most efficacious analgesics. But their efficacy is limited by side effects including mental clouding, tolerance, physical dependence, constipation and respiration depression (Benyamin et al., 2008). Therefore, there is a competitive advantage in developing a drug that is effective to inhibit pain but does not produce adverse effects.

Ideal target for pain management would be the receptors that are strictly located on nociceptive neurons. Targeting this type of receptors does not affect other neurons and, therefore, not impact other physiological functions. Alternative solution would be combining two or more drugs. Combination of multiple drugs for pain relief is used widely in clinical and animal studies (more than 3000 papers). A clinically useful combination could simply have additive or even subadditive analgesia, provided that there is less additivity for side effects (Gilron and Max, 2005). As opioids are effective for treatment of chronic pain (Bannwarth, 1999), combinations usually consist of opioid and others as adjunct. Unfortunately, most combinations produce significantly more frequent side effect-related trial dropouts than the drugs alone and the utility of such drug combinations is substantially limited (Chaparro et al., 2012). The present study demonstrated that a combination of ketanserin and propranolol produced synergistic antinociception. These two drugs are registered pharmaceutically and target two separate receptors in the periphery. The results showed that each drug produced anti-hyperalgesic effects in carrageenan-evoked model of inflammation. When these two drugs were given together at a minimal dose, they produced a significant inhibition on inflammatory hyperalgesia without tolerance.

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2. Materials and methods

2.1. Animals

The study was carried out using male Sprague-Dawley rats weighing 250–330 g. Animals were habituated to individual observation device for 30 min per day for a minimum of 3 days before the commencement of experiments. Care and treatment of animals were according to the guidelines for investigations of experimental pain in conscious animals and were approved by the Animal Care Committee at the Fujian Normal University. The experimenter was blind to the drug test conditions.

2.2. Models of inflammatory hyperalgesia

To make a model of carrageenan-evoked acute hyperalgesia, rats received an intraplantar (i.pl.) injection of carrageenan (2% in saline, 100 μl, Sigma Chemical Co., St. Louis, MO, USA) in the right hindpaw.

Inflammatory arthritis was induced with slightly modified method described previously (Schaible and Schmidt, 1985; Pozo et al., 1997; Sluka, 1998; Zhang et al., 2002). Briefly, the rat was anesthetized with barbiturate (45 mg/kg, i.p.). A solution of kaolin (4% in distilled water, 0.1 ml) was injected into the synovial cavity of right knee joint, using a lateral approach. Then, the knee joint was flexed and extended within the normal range of motion at regular intervals for 15 min. After that, 0.1 ml of a 2% aqueous solution of carrageenan (in saline) was injected into the joint cavity. The knee joint was again manipulated by slightly rapid flexion and extension movements for 5 min.

The muscle-mediated chronic pain was evoked by a modified method of the previously described protocol (Sluka et al., 2002). The skin covering the lateral gastrocnemius muscle was shaved, and rats were briefly anesthetized with a gaseous mixture of halothane supplemented with oxygen. One lateral gastrocnemius muscle belly was injected with 100 μl of carrageenan. Five days later the same gastrocnemius muscle was re-injected using an identical injection protocol. As a control for the injection procedure itself, a separate group of eight animals were injected with saline at pH 7.4.

2.3. Assessment of nociceptive threshold

Paw withdrawal latency (PWL) to radiant heat was used to assess thermal nociceptive threshold. A decrease in the PWL to noxious radiant heat in knee joint inflammation is also indicative of secondary hyperalgesia and is used to assess arthritis (Coderre and Wall, 1988; Houghton et al., 1998; Sluka et al., 1998; Zhang et al., 2002). Thermal hyperalgesia was evaluated by using an analgesimeter (Plantar Test, Ugo Basile, Comerio-Varese, Italy). On the day of the test, each animal was placed in a plastic cage with a glass floor. The plantar surface of hind paw was exposed to a beam of radiant heat through the glass floor. The radiant heat source consisted of an infrared bulb. Bulb intensity was adjusted so that the control latency was 7–9 s (10–12 s for arthritic rats). A photoelectric cell detected light reflected from the paw and turned off the lamp when paw movement interrupted the reflected light. The PWL was automatically displayed to the nearest 0.1 s. The cut-off time was 20 s in order to avoid tissue damage. The average of the three trials with 2-min interval was determined.

Mechanical threshold was measured in the hindpaw using an automated von Frey type system (Dynamic Plantar Anesthesiometer 37400, Ugo Basile, Italy). Rats were placed on a metal mesh surface under a plastic enclosure. The stimulator unit was placed beneath the selected hindpaw with the filament below the plantar surface of the rat. A paw withdrawal threshold (PWT) was measured by applying an increasing force (measured in grams) using a stainless-steel filament (0.5 mm diameter). To start, the electrodynamic actuator unit lifted the filament and exerted a force. The force was increased at a rate of 2.5 g/s until the rat moved its paw. A force of 50 g for 30 s was used as a cut-off point to preclude possible damage to the paw. The force was measured three times at 2 min-interval to generate mean value.

2.4. Drugs

Carrageenan (lambda type IV), ketanserin tartrate, *N*-2-[4-(2-methoxyphenyl-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635), naloxone hydrochloride or methiodide and propranolol were purchased from Sigma (St. Louis, MO, U.S.A.). Kaolin was obtained from Shanghai (Shanghai Chemical Inc., Shanghai, China). The drugs were dissolved in sterile saline except ketanserin. Stock solution of ketanserin was prepared in 20% dimethylsulfoxide (DMSO, Shenggong, Shanghai, China). The stocks were stored at 4 °C and diluted to the working concentration with sterile saline immediately before the experiment. The concentration of DMSO in the vehicle used to inject ketanserin was 0.8%. The dose of naloxone hydrochloride (Machelska et al., 2003) or methiodide (Varamini et al., 2012) was determined according to the literature. The 5-HT and opioid receptor antagonists were injected i.pl. or subcutaneously (s.c.)

2.5. Statistics

To present amplitude of the changes of PWL or PWT, data were presented in real values or converted to a percentage of baseline value that was measured 10 min before carrageenan treatment. Data were expressed as the mean ± standard error of mean (SEM). To detect changes over time between multiple groups (treatment group × time), data were analyzed using a two-way ANOVA. A P value less than 0.05 denoted the presence of a statistically significant difference.

3. Results

3.1. Effect of i.pl. administration of WAY1000635 on the maintenance of carrageenan-evoked hyperalgesia

Intraplantar injections of carrageenan at 0 h and saline at 1 h produced thermal hyperalgesia in a time-dependent manner. The hyperalgesia started at 1 h, peaked at 3 h and then slowly retreated in 48 h (control, Fig. 1A). The 5-HT_{1A} antagonist WAY100635 administered i.pl. 1 h after carrageenan injection dose-dependently increased PWL at 24 h compared with control group. Fig. 1A illustrates that WAY100635 at a dose of 3 μg did not alter carrageenan-evoked hyperalgesia at all tested time points. However, 10 μg of WAY100635 produced an increase in PWL at 24 h after carrageenan injection. Interestingly, the PWL was prolonged to 122 ± 5% which was above the baseline, indicating a state of hypoalgesia ($P < 0.001$ vs. control, Fig. 1A). Following the treatment with 30 μg of WAY10036, the PWLs were 74 ± 4, 83 ± 5 and 163 ± 2% at 6, 8 and 24 h, respectively. These values were significantly different from those in control group at corresponding time points which were 51 ± 7, 62 ± 8 and 80 ± 6%, respectively ($P < 0.05$ or 0.001, Fig. 1A).

As WAY100635 produced delayed hypoalgesia 24 h after the carrageenan injection, the opioid receptor antagonist naloxone was injected s.c. to determine the involved mechanism. Carrageenan and WAY100635 (30 μg) were given i.pl. at 0 and 1 h, respectively. Then, saline, naloxone hydrochloride or naloxone methiodide was injected s.c. at 24 h. Fig. 1B showed that PWL was 152 ± 6 and 146 ± 8% at 24 and 24.3 h, respectively, in carrageenan/WAY100635/saline group.