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Synergistic antinociception of intrathecal sildenafil with clonidine in the rat formalin test

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ABSTRACT

Spinal sildenafil (phosphodiesterase 5 inhibitor) and clonidine (alpha-2 adrenoceptor agonist) have shown antinociception. The author examined the properties of drug interaction after concurrent administration of intrathecal sildenafil-clonidine, and further clarified the reciprocity of sildenafil and clonidine. Catheters were inserted into the intrathecal space of male Sprague-Dawley rats. The formalin test was used as a nociceptive test, which was induced by subcutaneous injection of 50 μl of 5% formalin solution into the hindpaw. The pharmacological interaction was characterized using an isobolographic analysis. Intrathecal sildenafil and clonidine dose-dependently suppressed the flinching response observed during phase 1 and phase 2 in the formalin test. Isobolographic analysis revealed a synergistic interaction after intrathecal delivery of sildenafil-clonidine in both phases. Intrathecal yohimbine antagonized the antinociceptive action of intrathecal sildenafil during both phases in the formalin test. However, intrathecal ODQ failed to antagonize the antinociceptive action of intrathecal clonidine. These results suggest that sildenafil and clonidine, and the mixture of the two are effective against acute pain and facilitated pain state at the spinal level. Furthermore, synergism was noted after delivery of sildenafil-clonidine mixture. The antinociception of sildenafil can be modulated by spinal alpha-2 adrenoceptor, while the effect of clonidine is independent on the guanyly cyclase.

1. Introduction

Several lines of evidence suggest for the involvement of cyclic guanosine monophosphate (cGMP) in central and peripheral antinociceptive action (Ferreira and Nakamura, 1979; Sousa and Prado, 2001). Results from such research have shown that intraplantar dibutyryl-cGMP and intrathecal 8-bromo-cGMP produced antinociception in inflammatory hyperalgesia and neuropathic rats, respectively (Ferreira and Nakamura, 1979; Sousa and Prado, 2001).

Biochemically, guanylyl cyclase catalyzes the formation of cGMP from GTP (guanosine triphosphate), leading to the synthesis of cGMP, whereas cGMP-specific phosphodiesterase catalyzes the hydrolysis of cGMP to GMP (Pyne et al., 1996). Accordingly, intracellular cGMP concentrations are regulated by the action of guanylyl cyclase and the rate of degradation by cGMP-specific phosphodiesterase (Beavo, 1995; Pyne et al., 1996).

Sildenafil (Viagra®) is a novel inhibitor of cGMP-specific phosphodiesterase 5, which has proven to be effective in the treatment of male erectile dysfunction (Boolell et al., 1996). Previous studies have shown that intrathecal sildenafil produced an antinociception in formalin-induced hyperalgesia, which is mediated through the nitric oxide (NO)-cGMP-protein kinase G (PKG)-potassium channels pathway or opioid receptors (Araiza-Saldaña et al., 2005; Yoon et al., 2008). And it is reported that intrathecal clonidine reduced both acute pain and tissue injury hyperalgesia (Yoon and Choi, 2003; Zeng et al., 2007) and the antinociception was mediated through spinal alpha-2 adrenoceptor (Khodayar et al., 2006). These observations suggest that sildenafil and clonidine may have a comparable property in the regulation of nociception at the spinal level, but the nature of pharmacological interaction between sildenafil and clonidine remains to be determined.

On the other hand, it has been reported that the inhibitory effect of clonidine was blocked by guanylyl cyclase inhibitor (Ge et al., 2006), which suggests the effect of clonidine may be related to guanylyl cyclase pathway. However, the effect of alpha-2 adrenoceptor on the activity of sildenafil has not been determined.

Therefore, the purpose of the present study was to evaluate the characteristics of the spinally mediated interaction between sildenafil and clonidine in the formalin-induced nociception, which is

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characterized by two different nociceptive states, acute nociception followed by a facilitated state. In addition, we examined the reciprocity between sildenafil and clonidine.

2. Materials and methods

2.1. Animal preparation

The studies were reviewed and approved by the Institutional Animal Care Committee, Research Institute of Medical Science, Chonnam National University.

Experiments were performed on 8 weeks old adult male Sprague-Dawley rats weighing 250–300 g. The animals were housed in groups of four, with free access to standard rat diet and tap water in a room under 12:12 h light/dark cycle. Each rat was implanted with an intrathecal catheter for drug or vehicle administration under enflurane anesthesia (Yaksh and Rudy, 1976). A saline-flushed polyethylene-10 tube was inserted into the rat's subarachnoid space through an incision of the atlantooccipital membrane. The caudal part of the catheter was gently placed at the lumbar enlargement (about 8.5 cm from the incision). The rostral part of the catheter was tunneled subcutaneously to the skull and secured with steel wire. The skin wound was closed with 3–0 silk sutures. After intrathecal catheterization, rats were housed in each cage. Those rats showing postsurgical neurological deficit were excluded from the study and killed immediately with an overdose of volatile anesthetics. All testing were performed 5 days after intrathecal catheterization.

2.2. Drugs

The following drugs were used in this study: sildenafil, clonidine hydrochloride (Sigma Aldrich Co., St. Louis, MO, USA), yohimbine hydrochloride (Sigma), and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ, Sigma). Sildenafil was provided by Korea Pfizer. Sildenafil and ODQ were dissolved in 20% dimethylsulfoxide (DMSO). Clonidine and yohimbine were dissolved in normal saline and distilled water, respectively. Intrathecal administration of these agents was performed using a hand-driven, gear-operated syringe pump. All drugs were delivered in a volume of 10 µl solution, followed by an additional 10 µl of normal saline to flush the catheter.

2.3. Nociceptive test

The formalin test was performed as a nociceptive behavioral study (Zeng et al., 2007). The animals were injected subcutaneously with 50 µl of 5% formalin solution into the plantar surface of the hind paw using a 30 gauge needle. Formalin injection produces characteristic pain behavior i.e., a rapid, brief flexion of the injected paw, which is defined as flinching. Such pain behavior was therefore quantified by periodically counting the incident of flinching of the injected paw. The number of flinching was counted for 1 min periods at 1 and 5 min and at 5 min intervals from 10 to 60 min. Formalin-induced flinching response was observed in a characteristic biphasic style. Hence, the 1–9 min period was defined as phase 1 (early phase) of the formalin test and the 10–60 min period as phase 2 (late phase). Immediately following the completion of the formalin test, the rats were killed using a volatile anesthetics overdose.

2.4. Experimental paradigm

On the day of behavioral study, the rats were placed in a restraint cylinder for 15–20 min to allow them to adapt to their surroundings. Rats were then randomly allocated into one of the drug-treatment groups. The control study was done with solvents according to the drug. All experiments were carried out by experienced researchers blind to the drug condition. Each rat was tested only once.

2.4.1. Effects of sildenafil and clonidine

As the first series of experiments, the antinociceptive effects of intrathecal sildenafil (1, 3, 10, 30 µg, $n=32$) and clonidine (1, 3, 10, 30 µg, $n=35$) were examined for flinching response during phase 1 and phase 2 in the formalin test. All three drugs were injected 10 min before formalin injection. ED_{50} values (effective dose producing a 50% reduction in control formalin response) for the two agents were calculated from the dose–response in accordance with each phase.

2.4.2. Drug interaction

The characteristics of drug interaction between sildenafil and clonidine in the formalin test were analyzed by isobolographic method (Zeng et al., 2007). This technique compares the combinations of doses of each of the two agents that are determined to be equipotent. Thus, phase 1 and phase 2 ED_{50} values of sildenafil and clonidine were obtained from the dose–response curves of the first series of experiments. Next, sildenafil and clonidine were intrathecally coadministered at a dose of the ED_{50} values and fractions (1/2, 1/4, 1/8) of ED_{50} of each drug, and then the formalin test was conducted 10 min after the delivery of the mixture. Phase 1 ($n=27$) and phase 2 ($n=25$) ED_{50} values of the mixture were then obtained from the dose–response curves of the combined drugs, and these dose combinations were used for plotting the isobologram. An isobologram was constructed by plotting the ED_{50} values of the single agents on the *X* and *Y* axes, respectively. The theoretical additive dose combination was then calculated. From the variance of the total dose, individual variances for

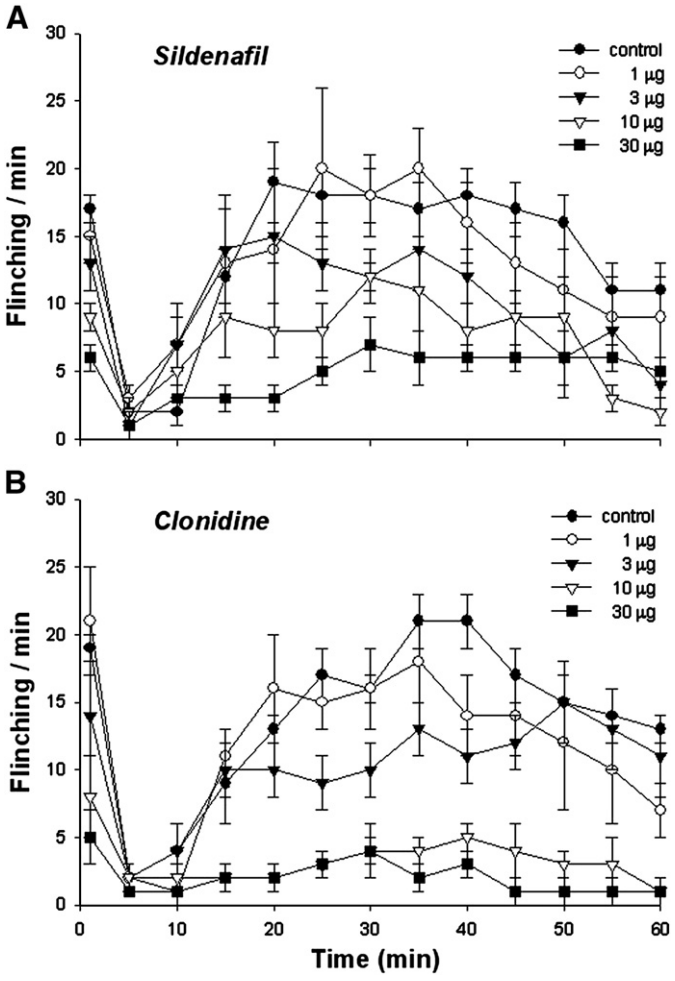

Fig. 1. Time effect curves of intrathecal sildenafil (A) and clonidine (B) for flinching in the formalin test. Each drug was administered 10 min before formalin injection. Formalin was injected at time 0. Data are presented as the number of flinching. Each point represents mean ± SEM of 6–7 rats.