Pharmacology, Biochemistry and Behavior 92 (2009) 583–588

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

Myung Ha Yoon [a,c,*], Chang Mo Kim [a], Hyung Gon Lee [a], Woong Mo Kim [a], Tae Hoon An [b], Yeo Ok Kim [a], Lan Ji Huang [c]

[a] Department of Anesthesiology and Pain Medicine, Chonnam National University, Medical School, Gwangju, South Korea
[b] Department of Anesthesiology and Pain Medicine, Chosun University, Medical School, Gwangju, South Korea
[c] The Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, Gwangju, South Korea

ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form 23 January 2009
Accepted 13 February 2009
Available online 28 February 2009

Keywords:
Antinociception
Clonidine
Sildenafil
Synergistic interaction
Alpha-2 adrenoceptor

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.

© 2009 Elsevier Inc. All rights reserved.

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

* Corresponding author. Department of Anesthesiology and Pain Medicine, Chonnam National University, Medical School, 8 Hakdong, Donggu, Gwangju 501-757, South Korea. Tel.: +82 62 220 6893; fax: +82 62 232 6294.
E-mail address: mhyoon@chonnam.ac.kr (M.H. Yoon).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.02.007

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

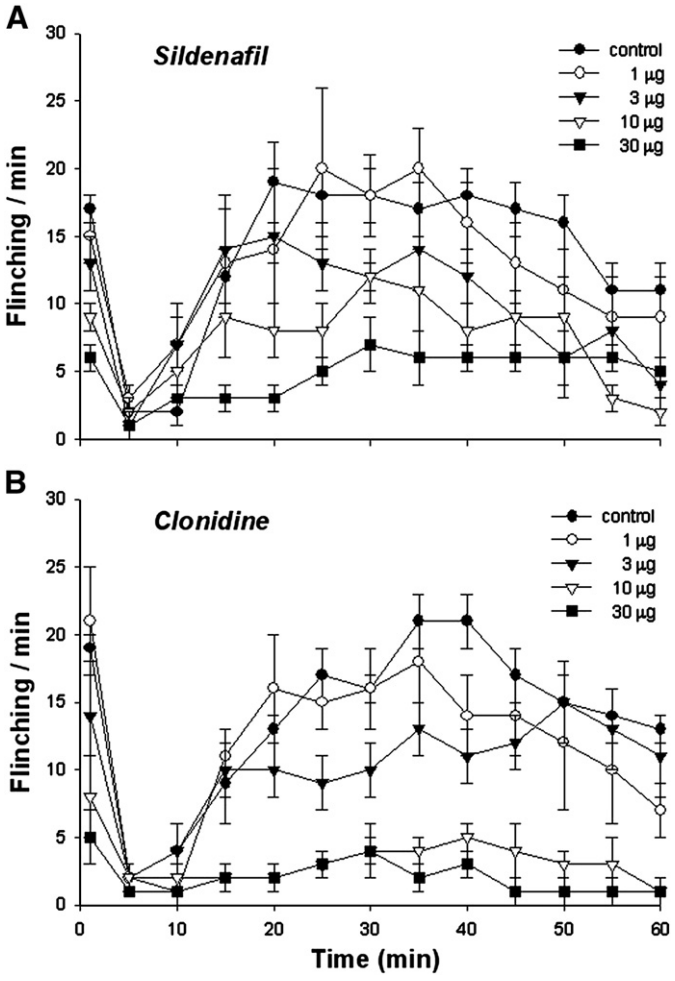

Fig. 1. Time effect curves of intrathecal sildenafil (A) and clonidine (B) for flinching in the formalin test. Each drug was administered 10 min before formalin injection. Formalin was injected at time 0. Data are presented as the number of flinching. Each point represents mean ± SEM of 6–7 rats.

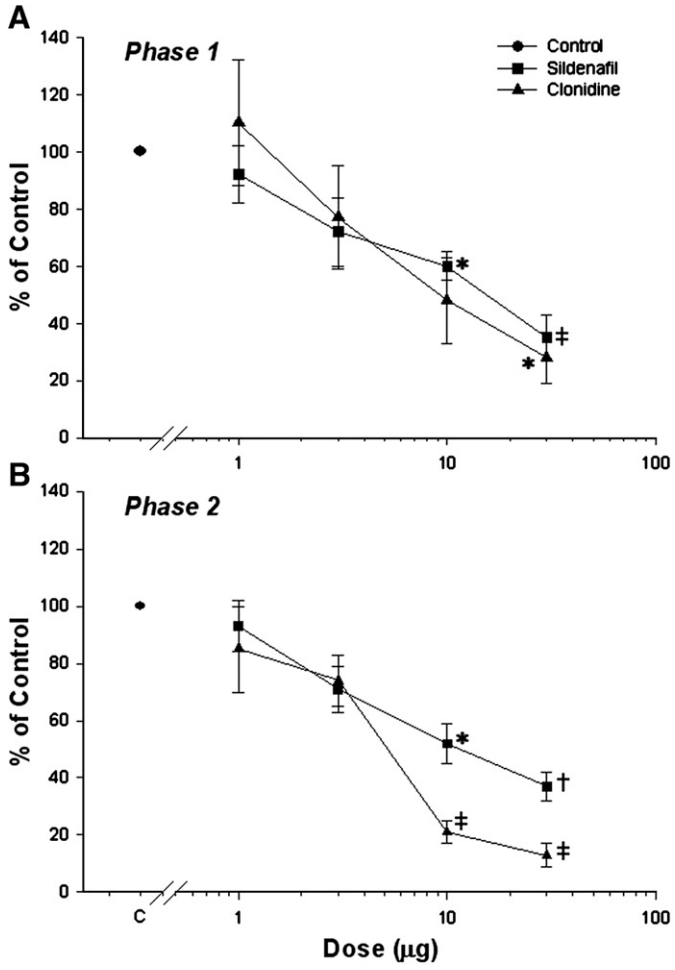

Fig. 2. Dose response curves of intrathecal sildenafil and clonidine for flinching during phase 1 (A) and phase 2 (B) in the formalin test. Data are presented as the percentage of control. Sildenafil and clonidine produced a dose-dependent inhibition of flinching response in both phases. Each point represents mean ± SEM of 6–7 rats. C = control. Compared with control, $^{*}p<0.05$, $^{\dagger}p<0.01$, $^{\ddagger}p<0.001$.

the agents in the combination were obtained. Additionally, to describe the magnitude of the interaction, a total fraction value was calculated according to the following formula:

$$\text{Total fraction value} = \frac{\text{ED}_{50} \text{ of drug 1 with drug 2}}{\text{ED}_{50} \text{ for drug 1 given alone}} + \frac{\text{ED}_{50} \text{ of drug 2 with drug 1}}{\text{ED}_{50} \text{ for drug 2 given alone}}$$

The fraction values indicate what portion of the single ED_{50} value was accounted for by the corresponding ED_{50} value for the combination. Values near 1 represent additivity. Values greater than 1 or less than 1 reflect an antagonism and a synergism, respectively.

2.4.3. Reciprocity of guanylyl cyclase and alpha-2 adrenoceptor

To determine whether the effect of intrathecal sildenafil was mediated through alpha-2 adrenoceptor or *vice versa*, alpha-2 adrenoceptor inhibitor (yohimbine 10 μg, $n=5$) or guanylyl cyclase inhibitor (ODQ 10 μg, $n=5$) were intrathecally administered 10 min before the delivery of sildenafil (30 μg) or clonidine (30 μg), respectively. The maximal doses of yohimbine and ODQ were determined from the pilot experiments or previous study (Araiza-Saldaña et al., 2005; Khodayar et al., 2006), and these doses were without affecting the control formalin response. The formalin test was conducted 10 min after administration of sildenafil or clonidine. These experiments were conducted in phase 1 and phase 2, respectively.

2.5. General behavior

In a separate experiment, additional rats ($n=20$) were estimated for any possible side effects such as motor impairment, sedation, and catalepsy with sildenafil (30 μg), clonidine (30 μg), and the mixture of sildenafil–clonidine (ED_{50}–ED_{50}). Behavioral examinations were carried out at 5, 10, 20, 30, 40, 50, and 60 min after delivery. Motor function was assessed by the placing-stepping reflex and the righting reflex (Yoon et al., 2003). Placing-stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of the table. Normal rats try to put the paw ahead into a position to walk. Righting reflex was evaluated by placing the rat horizontally with its back on the table. Normal rats give rise to an immediate and coordinated twisting of the body to an upright position. Moreover, pinna reflex and corneal reflex were evaluated with a paper string (Yoon et al., 2003). When a paper string stimulates the ear canal or the cornea, the rats normally shake their heads or blink, respectively. Changes in motor function were scored as follows: 0, normal; 1, slight deficit; 2,

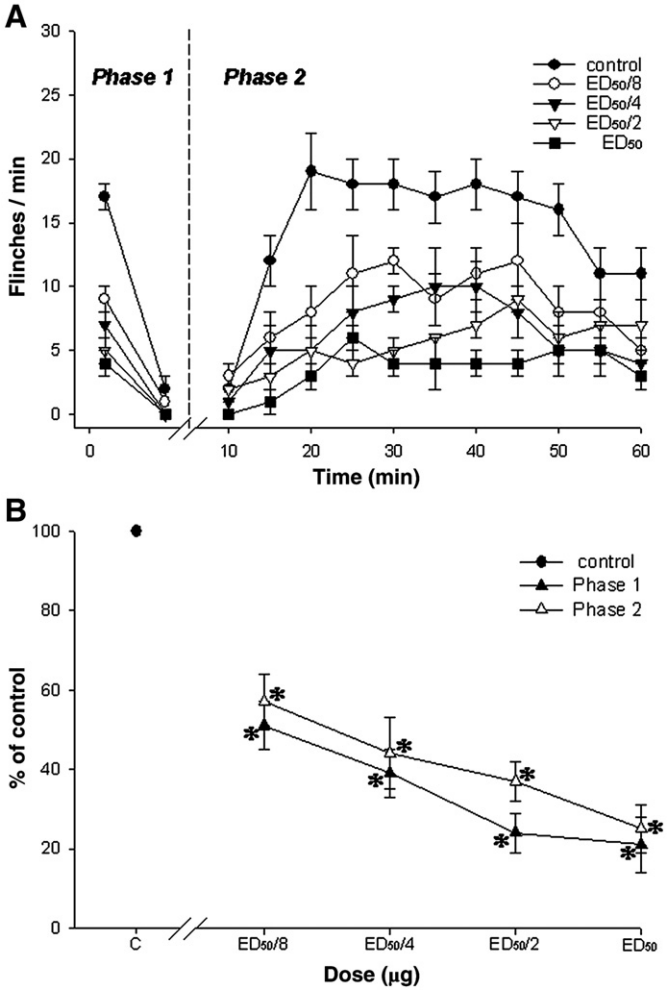

Fig. 3. Time effect curves (A) and dose response curves (B) of intrathecal mixture of sildenafil and clonidine for flinching in the formalin test. The mixture was administered 10 min before formalin injection. Formalin was injected at time 0. Data are presented as the number of flinching or the percentage of control. The mixture of sildenafil and clonidine produced a dose-dependent inhibition of flinching response in both phases. Each point represents mean ± SEM of 6–7 rats. C = control. Compared with control, $^{*}p<0.001$.

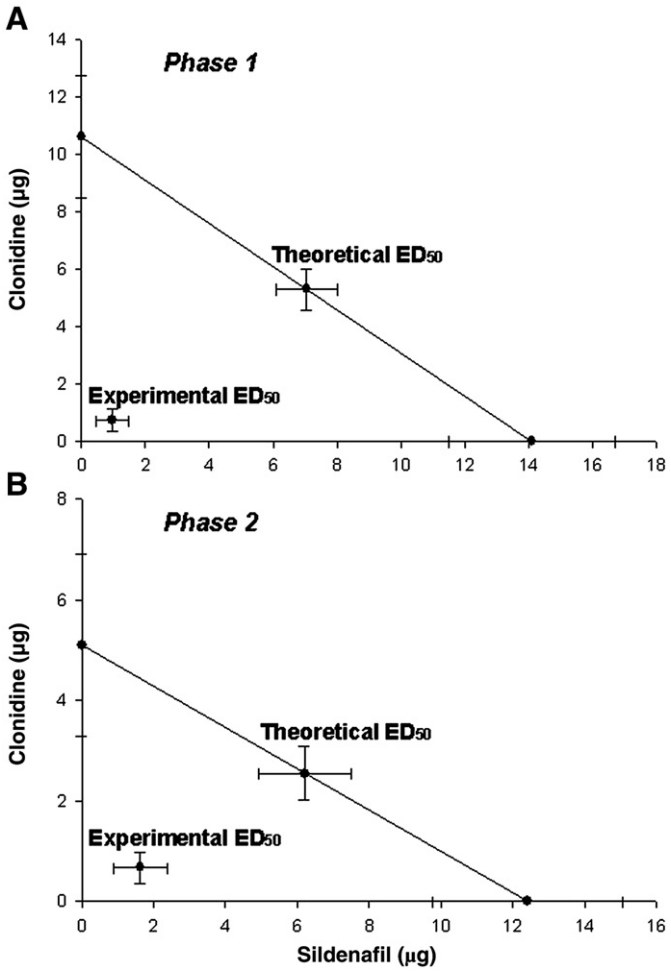

Fig. 4. Isobologram for the interaction between intrathecal sildenafil and clonidine during phase 1 (A) and phase 2 (B) in the formalin test. The ED50 values for each agent are plotted on the *x*- and *y*-axes, respectively. Horizontal and vertical bars indicate confidence intervals. The straight line connecting each ED50 value is the theoretical additive line, and the point on this line is the theoretical additive ED50. The experimental ED50 point was significantly different from the theoretical ED50 points, indicating a synergistic interaction.

moderate deficit; 3, severe deficit. Pinna reflex and corneal reflex were judged as present or absent.

2.6. Statistical analysis

Results are expressed as mean ± SEM. The time response data are presented as the number of flinching. The dose–response data are presented as percentage of control in each phase. To calculate the ED_{50} values of each drug, the number of flinching was converted to percentage of control according to the following formula:

$$\%\ \text{of control} = \frac{\text{Sum of phase 1(2) flinching count with drug}}{\text{Sum of control phase 1(2) fliching count}} \times 100$$

Dose–response data were analyzed by one-way analysis of variance (ANOVA) with least significant differences (LSD) for *post hoc*. The dose–response lines were fitted using least-squares linear regression and ED_{50}, and its 95% confidence intervals were calculated according to the method described by Tallarida (Tallarida, 2000).

The difference between theoretical ED_{50} and experimental ED_{50}, and the antagonism for the effects of sildenafil and clonidine were analyzed by *t*-test or one-way ANOVA with *post hoc* LSD, respectively. In all results statistical significance was considered at $p<0.05$.

3. Results

3.1. General behavior

The placing-stepping reflex and righting reflex were normal after intrathecal administration of sildenafil and clonidine, and the mixture

Table 1
ED_{50} values (μg) with 95% confidence intervals (CI) and total fraction value (TFV) of intrathecal agents.

Agents	ED_{50} (95% CI)		TFV	
	Phase 1	Phase 2	Phase 1	Phase 2
Sildenafil ($n=32$)	14.1 (6.8–29.6)	12.4 (7–21.8)		
Clonidine ($n=35$)	10.6 (4.5–24.8)	5 (3.3–7.7)		
Sildenafil[a] + Clonidine ($n=52$)	1.7 (0.8–3.7)	2.3 (1.1–4.7)	0.14	0.26

ED_{50}: effective dose needed to show a 50% inhibition of control formalin flinching response, *n*: number of rats.
[a] ED_{50} of sildenafil in a combination of sildenafil with clonidine.

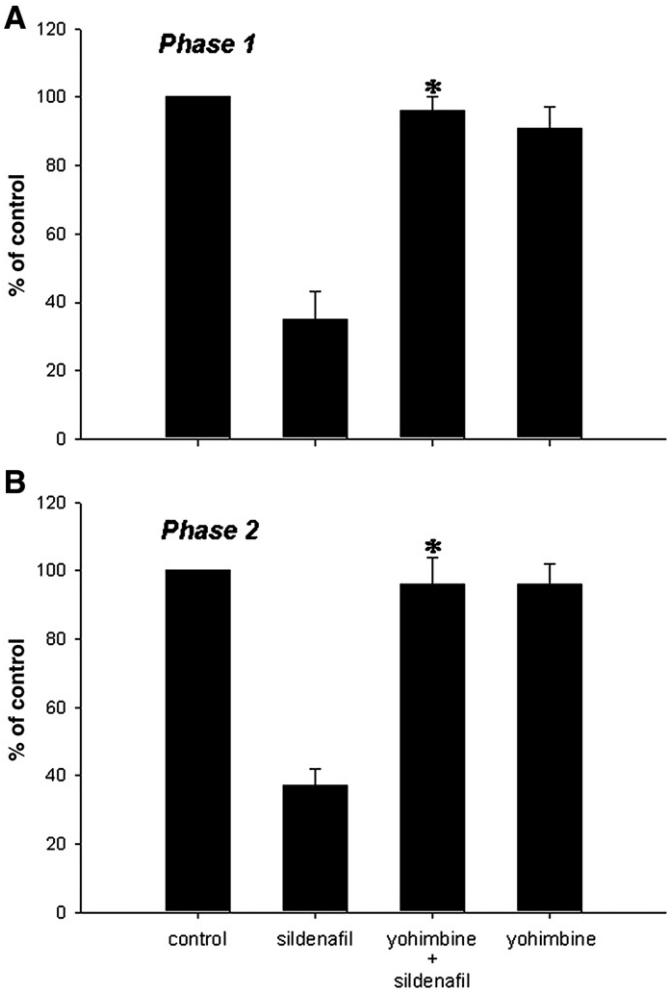

Fig. 5. Effect of intrathecal yohimbine (10 μg) for the antinociceptive action of intrathecal sildenafil (30 μg) during phase 1 (A) and phase 2 (B) in the formalin test. Yohimbine was administered 10 min before sildenafil, and the formalin test was conducted 10 min after sildenafil delivery. Data are presented as the percentage of control. Yohimbine alone did not affect the control response with formalin. The antinociceptive effect of sildenafil was reversed by yohimbine. Each column represents mean ± SEM of 5 rats. Compared with sildenafil *$p<0.001$.

of sildenafil–clonidine. Both pinna reflex and corneal reflex were present after intrathecal delivery of experimental drugs.

3.2. Effects of sildenafil and clonidine

Ipsilateral, but not contrateral, paw showed a biphasic flinching response after subcutaneous injection of formalin into the hindpaw. The time course effects of intrathecal sildenafil and clonidine, administered 10 min before formalin injection, are displayed in Fig. 1.

Intrathecal sildenafil [$F(4,27)=11.365$, $p<0.001$ in phase 1; $F(4,27)=16.431$, $p<0.001$ in phase 2] and clonidine [$F(4,30)=5.285$, $p<0.01$ in phase 1; $F(4,30)=22.316$, $p<0.001$ in phase 2] produced a dose-dependent suppression of the flinching response during phase 1 and phase 2 in the formalin test (Fig. 2A, B).

3.3. Drug interaction

Intrathecal coadministration of sildenafil and clonidine dose-dependently decreased the flinching response during phase 1 [$F(4,29)=39.705$, $p<0.001$] and phase 2 [$F(4,27)=11.365$, $p<0.001$] in the formalin test (Fig. 3A, B). Isobolographic analysis revealed a synergistic interaction between intrathecal sildenafil and clonidine during phase 1 ($df=76$, $t=4.32$, $p<0.001$) and phase 2 ($df=74$, $t=3.34$, $p<0.005$) in the formalin test (Fig. 4A, B). Thus, the experimental ED_{50} values were significantly lower than the calculated ED_{50} values. Total fraction value for the mixture of sildenafil and clonidine in phase 1 and phase 2 are described in Table 1.

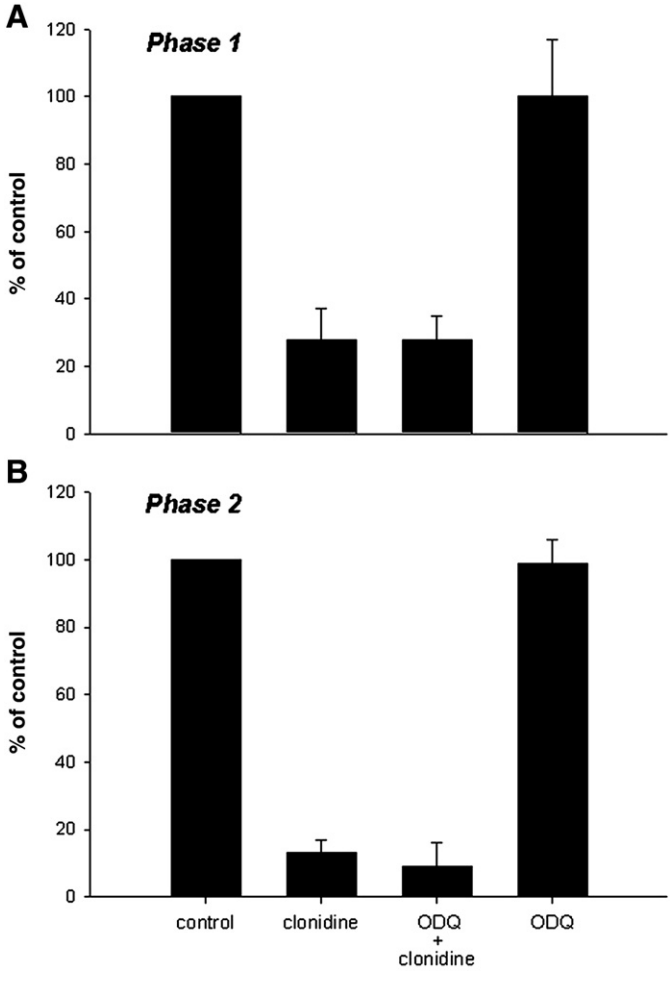

Fig. 6. Effects of intrathecal ODQ (10 μg) for the antinociceptive action of intrathecal clonidine (30 μg) during phase 1 (A) and phase 2 (B) in the formalin test. ODQ was administered 10 min before clonidine, and the formalin test was conducted 10 min after clonidine delivery. Data are presented as the percentage of control. ODQ alone did not affect the control response with formalin. The antinociceptive effect of clonidine was not reversed by ODQ. Each column represents mean ± SEM of 5 rats.

3.4. Reciprocity of guanylyl cyclase and alpha-2 adrenoceptor

The antinociceptive effect of intrathecal sildenafil was reversed by intrathecal yohimbine during phase 1 [$F(3,18)=36.808$, $p<0.001$] and phase 2 [$F(3,18)=42.504$, $p<0.001$] in the formalin test (Fig. 5A, B). However, the antinociceptive effect of intrathecal clonidine was not reversed by intrathecal ODQ during both phases [$F(3,18)=27.761$, $p=0.97$ in phase 1; $F(3,18)=141.28$, $p=0.52$ in phase 2] of the formalin test (Fig. 6A, B).

4. Discussion

Formalin-induced pain behavior appears as a biphasic pattern, with an early (phase 1) response followed by a late (phase 2) response (Wheeler-Aceto and Cowan, 1991). The phase 1 response is believed to represent a direct effect of formalin on sensory C fibers of primary afferent, thus the phase 1 of the formalin test reflects acute pain. On the other hand, the phase 2 response mirrors the sensitization during which inflammatory response develops (Hunskaar and Hole, 1977). And such sensitization may cause the activation of a wide dynamic range of dorsal horn neurons with very low levels of ongoing activity of primary afferents. Subsequently, the phase 2 response appears to be prominent and represents an intensified pain state in spite of a reduced level of afferent input, thus the phase 2 of the formalin test reflects a facilitated state. It is important to note that the phase 1 and phase 2 of the formalin test are not independent of each other. The phase 2 response is dependent on both the primary afferent input to initiate the sensitization and maintenance of afferent drive. If the phase 1 response is reduced, not only is there less sensitization, it implies that the afferent drive is reduced and there is less to magnify. Thus, it is assumed that the inhibition of the phase 1 is followed by the attenuation of the phase 2 response.

In the present study, intrathecal sildenafil inhibited the flinching response during phase 1 and phase 2 in the formalin test, which is consistent with previous findings (Araiza-Saldaña et al., 2005; Yoon et al., 2008). Interestingly, the ED_{50} of sildenafil on phase 2 was similar to that of phase 1. Furthermore, a two-fold lower ED_{50} for phase 2 was observed compared to that for phase 1 with clonidine. Previous study indicated that phase 1 block with intrathecal morphine resulted in marked attenuation of the facilitated state (Abram and Yaksh, 1993). These data jointly suggest that both sildenafil and clonidine work mainly by actions on the afferent drive and that all of the phase 2 effects are due to this.

Phosphodiesterase enzymes occur widely in biological systems (Beavo and Reifsnyder, 1990), which are involved in the hydrolysis of cGMP. Presently, eleven subfamilies of phosphodiesterase isoenzymes have been identified on the basis of their functional characteristics, such as substrate specificity, cellular distribution, and susceptibility to selective inhibitors (Francis et al., 2001; Ückert et al., 2006). It has been reported that phosphodiesterases 5, 6, and 9 are specific for cGMP hydrolysis and, in particular, phosphodiesterase 5 seems to be the most relevant enzyme (Pyne et al., 1996).

It has been proposed that cGMP may be responsible for antinociception (Ferreira and Nakamura, 1979; Sousa and Prado, 2001). This concept was based on several experimental findings. Local injection of dibutyryl-cGMP produced antinociception in a modification of the Randall–Selitto hyperalgesia (Ferreira and Nakamura, 1979). Furthermore, local sildenafil caused antinociception in carrageenan-induced hyperalgesia, the writhing test, and the second phase of the formalin test (Asomoza-Espinosa et al., 2001; Jain et al., 2001, 2003; Mixcoatl-Zecuatl et al., 2000; Patil et al., 2003). However, neither the phase 1 response in the formalin test nor the nociceptive thresholds in the tail-flick and hot-plate assays were affected by local

sildenafil (Asomoza-Espinosa et al., 2001; Jain et al., 2001; Mixcoatl-Zecuatl et al., 2000). In contrast to the local effect, intrathecal sildenafil attenuated not only acute pain but also the facilitated state in this study and previous experiments (Ferreira and Nakamura, 1979; Sousa and Prado, 2001). Furthermore, northern blot analysis revealed expression of phosphodiesterase 5 mRNA in the spinal cord (Loughney et al., 1998). Recently, neuroanatomical study also demonstrated cGMP-immunoreactivity presence and phosphodiesterase 5 mRNA expression in the spinal cord (de Vente et al., 2006). Thus, spinal sildenafil may be effective to the facilitated state as well as acute pain through the blockade of phosphodiesterase 5 isoenzyme in the spinal cord.

In the present study, intrathecal clonidine inhibited the flinching response during both phases in the formalin test, which agrees with previous data (Yoon and Choi, 2003; Zeng et al., 2007). Immunohistochemical and *in situ* hybridization studies indicate that alpha-2 adrenoceptor exists in the dorsal horn of the spinal cord, which is important area in the modulation of nociception (Pertovaara, 2006; Shi et al., 1999; Stone et al., 1998). The above observations suggest that intrathecal clonidine can directly act on the spinal alpha-2 adrenoceptor and produce an antinociceptive effect.

Isobolographic analysis conducted in this study showed synergistic interaction after concurrent delivery of intrathecal sildenafil–clonidine mixture during phase 1 and phase 2 in the formalin test. These results indicate that spinal sildenafil is able to enhance the antinociceptive effect of clonidine in acute pain and the facilitated state evoked by formalin injection. Although a pharmacological interaction between two kinds of drugs is most likely too complicated to define, several explanations may be possible. Firstly, drugs may interact by altering each others kinetics if two drugs have basically different mechanism of action. One agent may alter the actions of the other agents at its target sites. Hence, the action of sildenafil and clonidine may independently alter phosphodiesterase 5 enzyme and alpha-2 adrenoceptor, which leads to a synergistic interaction. Secondly, if mechanism of action of one drug may sequentially affect the action of another drug, a synergistic interaction may be expected. In this study, the antinociceptive effect of sildenafil was reversed by an alpha-2 adrenoceptor inhibitor. Therefore, these findings suggest that concurrent delivery of sildenafil and clonidine may further activate alpha-2 adrenoceptor, thereby leading to a synergism at the spinal level. Moreover, previous works indicated that the NO-cGMP-PKG-potassium channels pathway or opioid receptors are involved on sildenafil antinociception in the formalin model (Araiza-Saldaña et al., 2005; Yoon et al., 2008). Thus, sildenafil can produce an antinocieptive effect by modification of modulating a variety of systems which may alter the nociceptive processing. Such action may promote antinociception of other analgesics.

On the other hand, in the current study, the antinociception of intrathecal clonidine was not reversed by intrathecal guanylyl cyclase inhibitor, which suggests that the effect of clonidine may be guanylyl cyclase insensitive at the spinal level.

In summary, inhibition of phosphodiesterase 5 by intrathecal sildenafil alleviates acute pain and the facilitated state evoked by injection of formalin. Sildenafil interacts with clonidine in a synergistic manner at the spinal level. Furthermore, spinal alpha-2 adrenoceptor is involved in the antinociception of intrathecal sildenafil, while guanylyl cyclase is independent on the activity of clonidine at the spinal level.

References

Abram SE, Yaksh TL. Morphine, but not inhalation anesthesia, blocks post-injury facilitation. The role of preemptive suppression of afferent transmission. Anesthesiology 1993;78:713–21.

Araiza-Saldaña CI, Reyes-García G, Bermúdez-Ocaña DY, Pérez-Severiano F, Granados-Soto V. Effect of diabetes on the mechanisms of intrathecal antinociception of sildenafil in rats. Eur J Pharmacol 2005;527:60–70.

Asomoza-Espinosa R, Alonso-Lopez R, Mixcoatl-Zecuatl T, Aguirre-Banuelos P, Torres-Lopez JE, Granados-Soto V. Sildenafil increases diclofenac antinociception in the formalin test. Eur J Pharmacol 2001;418:195–200.

Beavo JA. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol Rev 1995;75:725–48.

Beavo JA, Reifsnyder DH. Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. Trends Pharmacol Sci 1990;11:150–5.

Boolell M, Gepi-Attee S, Gingell JC, Allen MJ. Sildenafil, a novel effective oral therapy for male erectile dysfunction. Br J Urol 1996;78:257–61.

de Vente J, Markerink-van Ittersum M, Vles JS. The role of phosphodiesterase isoforms 2, 5, and 9 in the regulation of NO-dependent and NO-independent cGMP production in the rat cervical spinal cord. J Chem Neuroanat 2006;31:275–303.

Ferreira SH, Nakamura M. Prostaglandin hyperalgesia, a cAMP/Ca^{2+} dependent process. Prostaglandins 1979;18:179–90.

Francis SH, Turko IV, Corbin JD. Cyclic nucleotide phosphodiesterases: relating structure and function. Prog Nucleic Acid Res Mol Biol 2001;65:1-52.

Ge YX, Xin WJ, Hu NW, Zhang T, Xu JT, Liu XG. Clonidine depresses LTP of C-fiber evoked field potentials in spinal dorsal horn via NO-cGMP pathway. Brain Res 2006;1118:58–65.

Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1977;30:103–14.

Jain NK, Patil CS, Singh A, Kulkarni SK. Sildenafil-induced peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. Brain Res 2001;909:170–8.

Jain NK, Patil CS, Singh A, Kulkarni SK. Sildenafil, a phosphodiesterase-5 inhibitor, enhances the antinociceptive effect of morphine. Pharmacology 2003;67:150–6.

Khodayar MJ, Shafaghi B, Naderi N, Zarrindast MR. Antinociceptive effect of spinally administered cannabinergic and 2-adrenoceptor drugs on the formalin test in rat: possible interactions. J Psychopharmacol 2006;20:67–74.

Loughney K, Hill TR, Florio VA, Uher L, Rosman GJ, Wolda SL, et al. Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. Gene 1998;216:139–47.

Mixcoatl-Zecuatl T, Aguirre-Banuelos P, Granados-Soto V. Sildenafil produces antinociception and increases morphine antinociception in the formalin test. Eur J Pharmacol 2000;400:81–7.

Patil CS, Jain NK, Singh A, Kulkarni SK. Modulatory effect of cyclooxygenase inhibitors on sildenafil-induced antinociception. Pharmacology 2003;69:183–9.

Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol 2006;80:53–83.

Pyne NJ, Arshavsky V, Lochhead A. cGMP signal termination. Biochem Soc Trans 1996;24:1019–22.

Shi TJ, Winzer-Serhan U, Leslie F, Hökfelt T. Distribution of alpha2-adrenoceptor mRNAs in the rat lumbar spinal cord in normal and axotomized rats. Neuroreport 1999;10:2835–9.

Sousa AM, Prado WA. The dual effect of a nitric oxide donor in nociception. Brain Res 2001;897:9-19.

Stone LS, Broberger C, Vulchanova L, Wilcox GL, Hokfelt T, Riedl MS, et al. Differential distribution of alpha2A and alpha2C adrenergic receptor immunoreactivity in the rat spinal cord. J Neurosci 1998;18:5928–37.

Tallarida RJ. Drug synergism and dose-effect data analysis. 1st. Ed. New York: Chapman & Hall/CRC; 2000. p. 21–71.

Ückert S, Hedlund P, Andersson KE, Truss MC, Jonas U, Stief CG. Update on phosphodiesterase (PDE) isoenzymes as pharmacologic targets in urology: present and future. Eur Urol 2006;50:1194–207.

Wheeler-Aceto H, Cowan A. Neurogenic and tissue-mediated components of formalin-induced oedema: evidence for supraspinal regulation. Agents Actions 1991;34:264–9.

Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yoon MH, Choi JI. Pharmacologic interaction between cannabinoid and either clonidine or neostigmine in the rat formalin test. Anesthesiology 2003;99:701–7.

Yoon MH, Choi JI, Jeong SW. Antinociception of intrathecal cholinesterase inhibitors and cholinergic receptors in rats. Acta Anaesthesiol Scand 2003;47:1079–84.

Yoon MH, Kim WM, Lee HG, Kim YO, Huang LJ, An TH. Roles of opioid receptor subtypes on the antinociceptive effect of intrathecal sildenafil in the formalin test of rats. Neurosci Lett 2008;441:125–8.

Zeng W, Chen X, Dohi S. Antinociceptive synergistic interaction between clonidine and ouabain on thermal nociceptive tests in the rat. J Pain 2007;8:983–8.